DMT-TIC DI- AND TRI-PEPTIDIC DERIVATIVES AND RELATED COMPOSITIONS AND METHODS OF USE

CROSS-REFERENCE TO RELATED PATENT APPLICATION

This patent application claims the benefit of U.S. Provisional Patent Application No. 60/192,128, which was filed on March 24, 2000.

TECHNICAL FIELD OF THE INVENTION

The present invention relates to di- and tri-peptidic derivatives comprising the pharmacophore Dmt-Tic and related compositions and methods of use in the antagonism and agonism of δ and μ opioid receptors and in the inhibition of P-glycoprotein, specifically hMDR-1.

BACKGROUND OF THE INVENTION

Endogenous opioids are believed to be involved in the modulation of pain perception, in mood and behavior, learning and memory, diverse neuroendocrine functions, immune regulation and cardiovascular and respiratory function. Opioids also have a wide range of therapeutic utilities, such as treatment of opiate and alcohol abuse, neurological diseases, neuropeptide or neurotransmitter imbalances, neurological and immune system dysfunctions, graft rejections, pain control, shock and brain injuries.

There are believed to be three types of opiate receptors, namely δ, κ and μ. Genes encoding these three main receptor types now have been cloned. Sequencing of the cloned opioid receptor genes has revealed a substantial degree of amino acid homology between different receptor types (Meng et al., *PNAS USA* 90: 9954-9958 (1993); Thompson et al., *Neuron* 11: 903-913 (1993); Evans et al., *Science* 258: 1952-1955 (1992); and Kieffer et al., *PNAS USA* 89: 12048-12052 (1992)), which explains the tendency of opioid receptor ligands, even those reported to be selective, to bind to more than one type of opioid receptor. Based on differences in the binding profiles of natural and synthetic ligands, subtypes of opioid receptors have been suggested, including μ1 and μ2 (Pasternak et al., *Life Sci.* 38: 1889-1898 (1986)) and κ1 and κ2 (Zukin et al., *PNAS USA* 85: 4061-4065 (1988)). Different subtypes of a given type of opioid receptor may co-exist in a single cell (Evans et al. (1992), *supra*; and Kieffer et al. (1992), *supra*).

The μ opioid receptor in the brain appears to mediate analgesia (Kosterlitz et al., *Br. J. Pharmacol.* 68: 333-342 (1980)). It is also believed to be involved with other undesirable effects, such as respiratory depression (Ward et al., *Soc. Neurosci. Symp.* 8: 388 (abstract) (1982)), suppression of the immune system (Plotnikoff et al., *Enkephalins and Endorphins: Stress and the Immune System*, Plenum Press, NY (1986); Yahya et al., *Life Sci.* 41: 2503-2510 (1987)) and addiction (Roemer et al., *Life Sci.* 27: 971-978 (1981)). Its side effects in the periphery include inhibition of intestinal motility (Ward et al., *Eur. J. Pharmacol.* 85: 163-170 (1982)) and secretion in the small intestine (Coupar, *Br. J. Pharmacol.* 80: 371-376 (1983)).

δ-opioid receptors also mediate analgesic but are not involved in addiction. They may have an indirect role in immune suppression.

There appears to be a single binding site for agonists and antagonists in the ligand-binding domain of δ receptors. Thus, the "message domain" of δ-agonists and δ-antagonists probably presents a similar low energy conformer in order to fit the receptor cavity. The minimum size of that "message domain" constitutes the dimensions of a dipeptide (Temussi et al., *Biochem. Biophys. Res. Commun.* 198: 933-939 (1994); Mosberg et al., *Lett. Pept. Sci.* 1: 69-72 (1994); and Salvadori et al., *J. Med. Chem.* 42: 3100-3108 (1997)), which has a specific spatial geometry in solution (Bryant et al., *Trends Pharmacol. Sci.* 18: 42-46 (1998); Bryant et al., *Biol. Chem.* 378: 107-114 (1997); Crescenzi et al., *Eur. J. Biochem.* 247: 66-73 (1997); and Guerrini et al., *Bioorg. Med. Chem.* 6: 57-62 (1998)) as seen in the crystallographic evidence for TIPP analogues (Flippen-Anderson et al., *J. Pept. Res.* 49: 384-393 (1997)) and *N,N*$(Me)_2$-Dmt-Tic-OH.

The Dmt-Tic pharmacophore represents a distinct class of δ-opioid antagonists (Salvadori et al., *Mol. Med.* 1: 678-689 (1995); Bryant et al. (1998), *supra*; and Lazarus et al., *Drug Dev. Today* 1998: 284-294). Observations of differences between the δ opioid receptor binding of Dmt-Tic peptides and their Tyr-Tic cognates (Salvadori et al. (1995), *supra*; Lazarus et al. (1998), *supra*; and Lazarus et al., *Int'l Symp. on Peptide Chem. and Biol.*, Changchung, PRC (1999)) indicates that Dmt assumes a predominant role in the alignment or anchoring of the peptide within δ, μ and κ opioid receptor binding sites (Bryant et al. (1998), *supra*; and Bryant et al. (1997), *supra*; Crescenzi et al. (1997), *supra*; and Guerrini et al. (1998), *supra*) or affects the conformation of the dipeptide antagonists in solution (Bryant et al. (1997), *supra*; and Crescenzi et al. (1997), *supra*). Furthermore,

observations of differences between the spectra of activity exhibited by the Tyr-Tic cognates of certain Dmt-Tic peptides (Schiller et al., *PNAS USA* 89: 11871-11875 (1992); Schiller et al., *J. Med. Chem.* 36: 3182-3187 (1993); Schiller et al., *Peptides* Hodges and Smith, eds., ESCOM (1994); pp. 483-486; Temussi et al. (1994), *supra*; Mosberg et al. (1994), *supra*; Salvadori et al. (1995), *supra*; Lazarus et al. (1998), *supra*; and Lazarus et al. (1999), *supra*) and the corresponding Dmt-Tic peptides suggests that the C-terminal "address" portion of the peptide can influence the "message domain."

Recently, cyclic peptides and di- and tri-peptides comprising the pharmacophore Dmt-Tic have been developed and have been shown to exhibit high selectivity, affinity and potency for the δ-opioid receptor. Such peptides have been shown to function as either agonists, partial agonists, antagonists, partial antagonists or mixed antagonists/agonists for opioid receptors (see Lazarus et al., U.S. Patent No. 5,780,589, and Schiller, U.S. Patent No. 5,811,400).

The uniqueness of the δ receptor has led to the use of moderately δ-selective alkaloid antagonists in clinical trials, such as for the amelioration of the effects of alcoholism (Froehlich et al., *Alcohol. Clin. Exp. Res.* 20: A181-A186 (1996)), the treatment of autism (Lensing et al., *Neuropsychobiol.* 31: 16-23 (1995)), and Tourette's syndrome (Chappell, *Lancet* 343: 556 (1994)). The δ-opiate antagonist naltrindole (Portoghese et al., *Eur. J. Pharm.* 146: 185-186 (1998)) has been shown to inhibit the reinforcing properties of cocaine (Menkens et al., *Eur. J. Pharm.* 219: 346-346 (1992)), to moderate the behavioral effects of amphetamines (Jones et al., *J. Pharmacol. Exp. Ther.* 262: 638-645 (1992)), and to suppress the immune system (Jones et al. (1992), *supra*) for successful organ transplantation (House et al., *Neurosci. Lett.* 198: 119-122 (1995)) in animal models (Arakawa et al., *Transplant Proc.* 24: 696-697 (1992); Arakawa et al., *Transplant* 53: 951-953 (1992); and Arakawa et al., *Transplant. Proc.* 25: 738-740 (1993)). The same effects also have been shown for 7-benzylspiroindanylnaltrexone (Lipper et al., *Eur. J. Pharmacol.* 354: R3-R5 (1998)).

The intractable membrane barriers, such as the blood-brain barrier (BBB), must be circumvented in order for peptide antagonists to express activity *in vivo* (Ermisch et al., *Physiol. Rev.* 73: 489-527 (1993)). The requisite physicochemical properties of compounds capable of passing through this barrier include low molecular weight (< 800 Da) and high octanol-water coefficient characteristics.

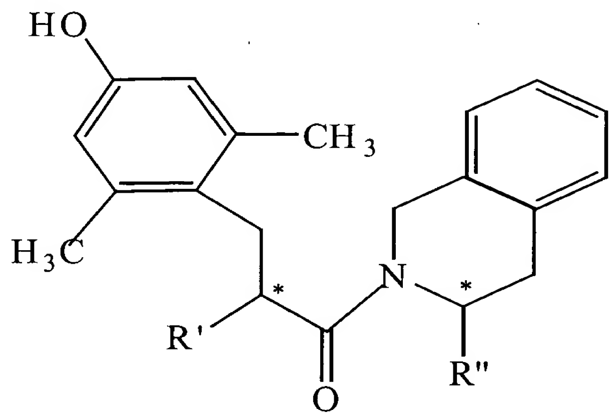
In view of the above, the present invention seeks to provide more potent δ-opioid antagonists and δ-opioid antagonists with high dual binding affinity and biological activity toward δ-opioid and μ-opioid receptors.

In addition to the above, one of the major chemical determinants for the inhibition of hMDR-1 involves the presence of strong hydrophobic substituents necessary for lipid solubility (Ford et al., *Cancer Res.* 50: 1748-1756 (1990); Zamora et al., *Mol. Pharmacol.* 33: 454-462 (1988); and Nogae et al., *Biochem. Pharmacol.* 38: 519-527 (1989)) as constituted by saturated and aromatic rings, as well as a tertiary nitrogen (Zamora et al. (1988), *supra*; Ramu et al., *Int. J. Cancer* 43: 487-491 (1989); and Pearce et al., *PNAS USA* 86: 5128-5133 (1989)). In addition to a role in drug resistance, the human Pgp-1 protein is expressed in a variety of normal human tissues and plays an important physiological role in maintenance of the BBB (Schinkel et al., *J. Clin. Invest.* 97: 2517-2524 (1996); Schinkel et al., *Int. J. Clin. Pharmacol. Ther.* 36: 9-13 (1998); and Jonker et al., *Br. J. Pharmacol.* 127: 43-50 (1999)) . In the BBB, Pgp activity in apical membranes of the capillary endothelial cells functions to at least partially exclude a wide variety of hydrophobic toxicants from the brain (Zamora et al. (1988), *supra*; Schinkel et al. (1996), *supra*; and Jonker et al., *Br. J. Pharmacol.* 127: 43-50 (1999)). These include some drugs with central nervous system activities, such as certain opiate alkaloids and analogues thereof (Callaghan et al., *J. Biol. Chem.* 268: 16059-16064 (1993)). Thus, the present invention also seeks to provide inhibitors of hMDR-1.

These and other objects of the present invention, as well as additional inventive features, will be apparent to the ordinarily skilled artisan from the detailed description provided herein.

BRIEF SUMMARY OF THE INVENTION

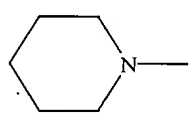
The present invention provides a compound of formula:



wherein R' is selected from the group consisting of

H_2N—,

H_2NH_2C-,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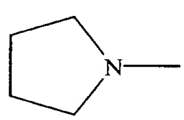
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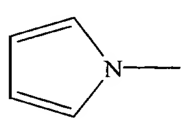
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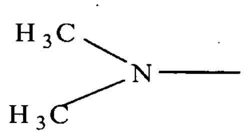
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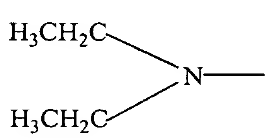
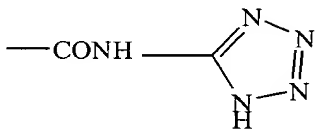

and

R" is selected from the group consisting of

—CH_2COOH,

$-COOH$,

$-CONHNH_2$,



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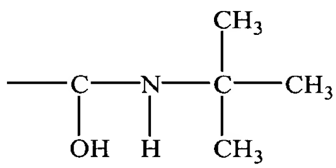

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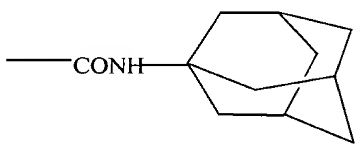
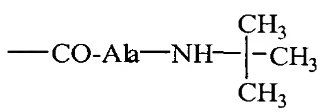


,

$-$CO-Ala$-NHCH_3$,



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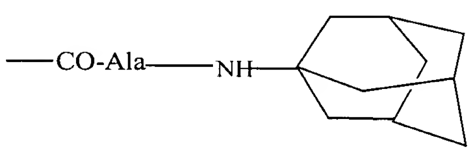

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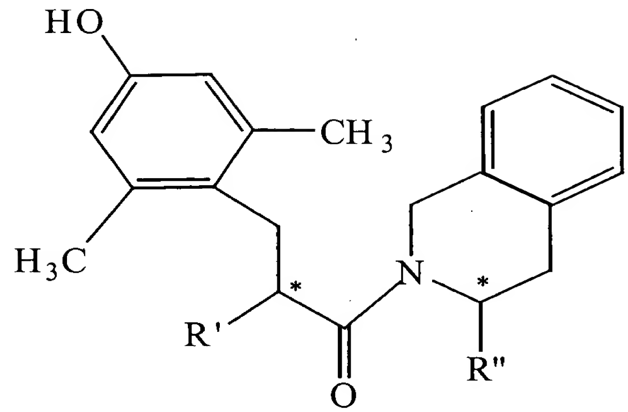
Also provided by the present invention is a composition comprising at least one compound of the above formula.

The present invention further provides methods of treatment. In one embodiment, a method of treating a mammal in need of an antagonist of a δ-opioid receptor is provided. The method comprises administering at least one compound of formula:

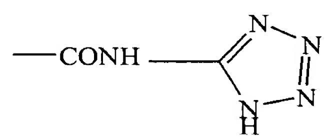


wherein (i) R' is H_2N— and

R" is —CH_2COOH ,

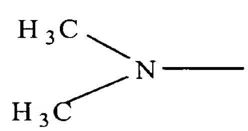
(ii) R' is H_2N— and

R" is



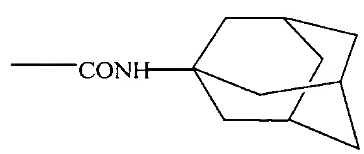
,

(iii) R' is



and

R" is



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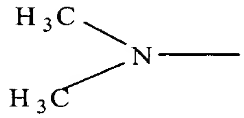
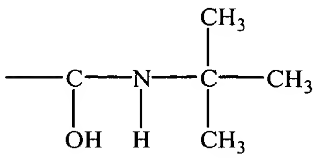
(iv) R' is



and

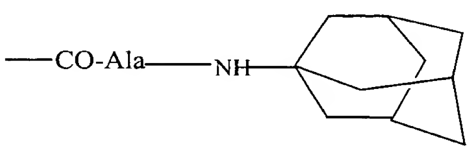
R" is



,

(v) R' is H_2N— and

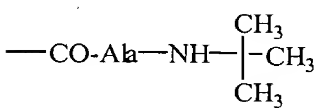
R" is



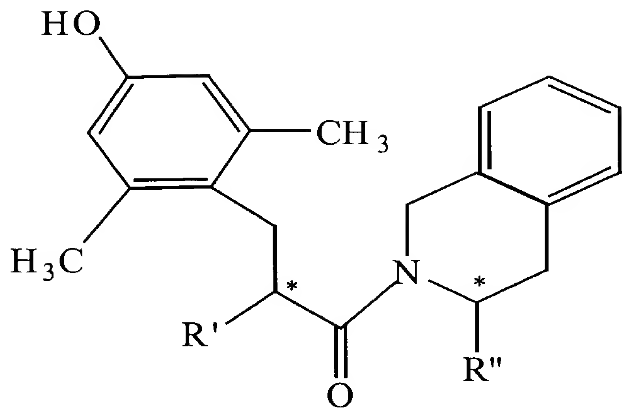
,

or (vi) R' is H_2N— and

R" is

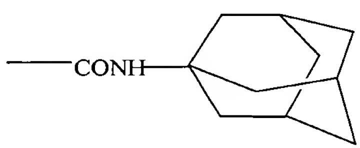


in an amount that antagonizes a δ-opioid receptor in the mammal. In another embodiment, the present invention may provide a method of treating a mammal in need of an agonist of a δ-opioid receptor. The method may comprise administering at least one compound of formula:



wherein (i) R' is H_2N— and

R'' is

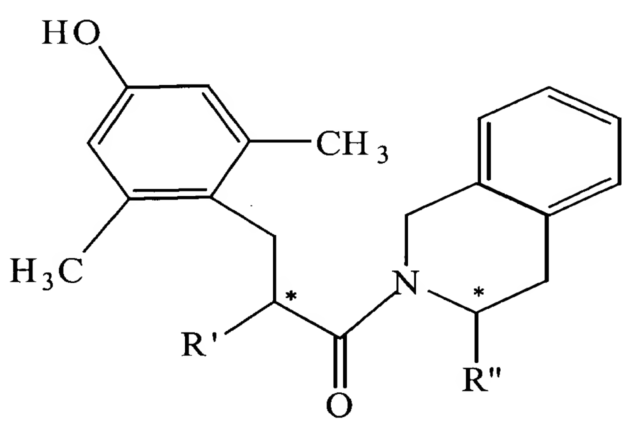


or

(ii) R' is H_2N— and

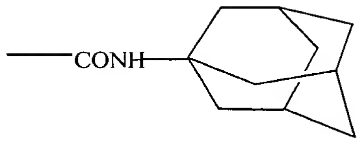
R'' is —CO-Ala—$NHCH_3$

in an amount that agonizes a δ-opioid receptor in the mammal. In yet another embodiment, the present invention may provide a method of treating a mammal in need of an agonist of a μ-opioid receptor. The method may comprise administering at least one compound of formula:



wherein (i) R' is H_2N— and

R" is



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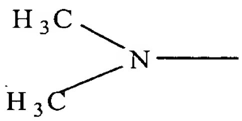
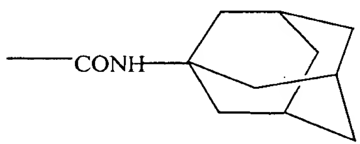
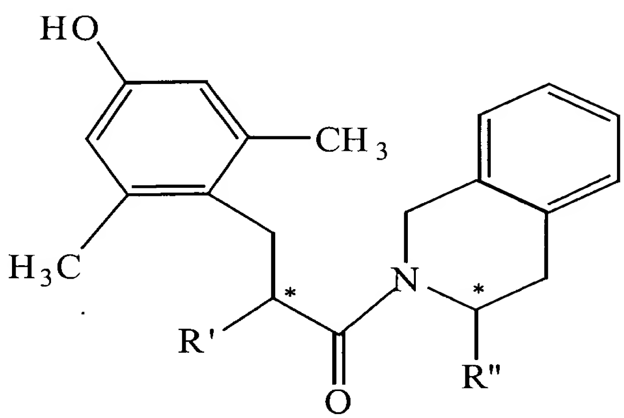
(ii) R' is  and

R" is

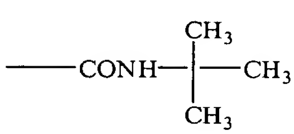


or (iii) R' is H_2N— and R" is —CO-Ala—$NHCH_3$ in an amount that agonizes a μ-opioid receptor in the mammal. In still yet another embodiment, the present invention provides a method of inhibiting the binding of an opioid receptor-binding compound with a P glycoprotein in a mammal. The method comprises administering at least one compound of formula:



wherein (i) R' is H_2N— and

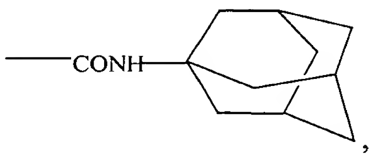
R" is



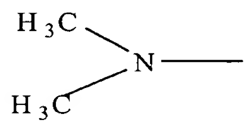
,

(ii) R' is H_2N— and

R'' is

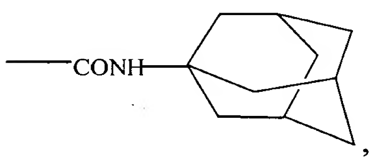


(iii) R' is



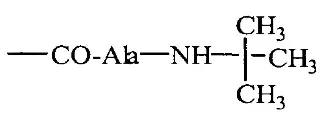
and

R'' is



(iv) R' is H_2N— and

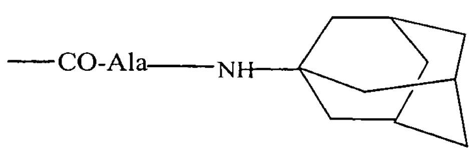
R'' is



or

(v) R' is H_2N— and

R'' is



in an amount effective to inhibit the binding of an opioid receptor-binding compound with a P glycoprotein in a mammal.

BRIEF DESCRIPTION OF THE FIGURES

Fig. 1A is a graph of fluorescence vs. concentration (μM).

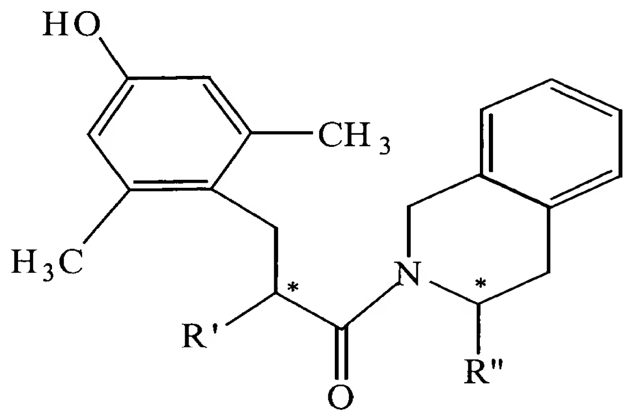
Fig. 1B is a graph of fluorescence vs. concentration (μM).

Fig. 1C is a graph of fluorescence vs. concentration (μM).

Fig. 1D is a graph of fluorescence vs. concentration (μM).

DETAILED DESCRIPTION OF THE INVENTION

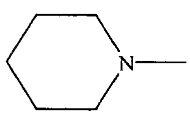
The present invention provides a compound of formula:



wherein R' is selected from the group consisting of

H_2N—,

H_2NH_2C-,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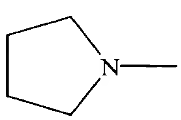
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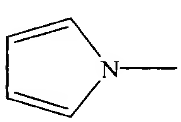
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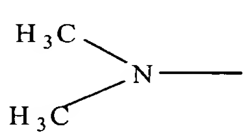
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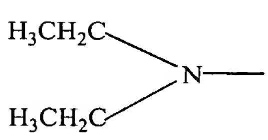
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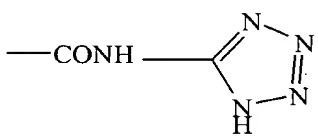
R" is $-CH_2COOH$,

$-COOH$,

$-CONHNH_2$,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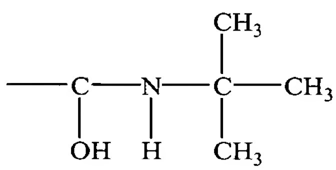
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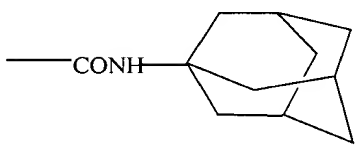
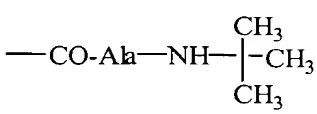

,

$-CO\text{-}Ala-NHCH_3$,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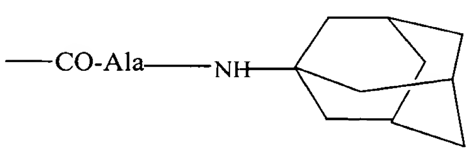
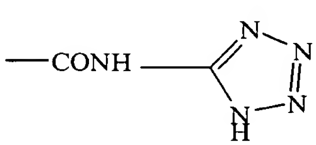
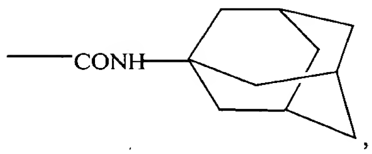
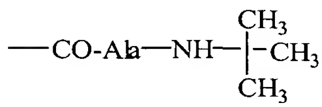

.

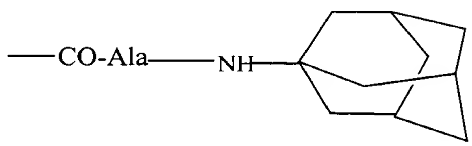
When R' is H_2N-, R" can be $-CH_2COOH$, $-CONHNH_2$,


,

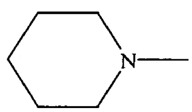
,

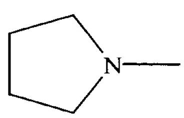
—CO-Ala—NHCH$_3$,

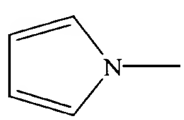
, or

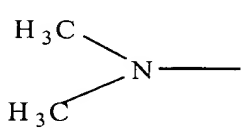
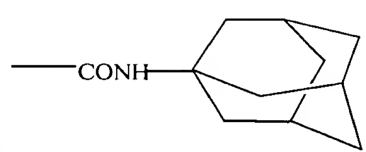
.

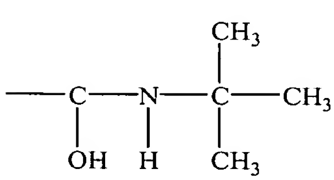
When R' is H_2NH_2C-, R'' can be —COOH.

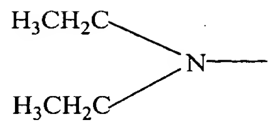
When R' is , R'' can be —COOH.

When R' is , R'' can be —COOH.

When R' is , R'' is —COOH.

When R' is , R'' can be  or

.

When R' is  , R'' can be —COOH.

The present inventive compounds can be synthesized by standard methods known to those of ordinary skill in the art. See, for example, *Modern Techniques of Peptide and Amino Acid Analysis*, John Wiley & Sons (1981); Bodansky, *Principles of Peptide Synthesis*, Springer Verlag (1984)). Specific examples of the synthesis of the present inventive compounds are set forth in the Examples herein.

Once the desired compound is synthesized, it can be isolated from the reaction mixture and purified by a variety of standard methods. Examples of isolation and purification methods include, but are not limited to, crystallization, HPLC, affinity chromatography and open column silica chromatography. Specific examples of the isolation and purification of the present inventive compounds are set forth in the Examples herein.

Whether an above-described compound functions as an agonist, a partial agonist, an antagonist, a partial antagonist, or a mixed agonist/antagonist is set forth in part in the Examples herein. Additionally, conventional techniques known to those of ordinary skill in the art can be used to make such determinations. Examples of such techniques include, but are not limited to, the mouse vas deferens *in vitro* assay of δ receptors and the guinea pig ileum *in vitro* assay of μ receptors as described in the Examples. Examples of *in vivo* studies include, but are not limited to, the tail flick test (Harris et al., *J. Pharmacol. Meth.* 20: 103-108 (1988); and Sing et al., *P.A. Amber* (v. 3.0. rev. A), Dept. Pharm. Chem., University of California, San Francisco (1988)).

The present invention further provides a composition comprising at least one of the above compounds. Desirably, the composition comprises at least one carrier, which is preferably a pharmaceutically acceptable carrier, diluent or vehicle. Also, desirably, the composition is formulated for human administration. Pharmaceutically acceptable carriers are well-known to those of ordinary skill in the art, as are suitable methods of administration. The choice of carrier will be determined, in part, by the particular method used to administer the composition. One of ordinary skill in the art will also appreciate that various routes of administering a composition are available, and, although more than one route can be used for administration, a particular route can provide a more immediate and

more effective reaction than another route. Accordingly, there are a wide variety of suitable formulations of compositions that can be used in the present inventive methods.

A compound of the present invention can be made into a formulation suitable for parenteral administration, preferably intraperitoneal administration, or dural administration. Such a formulation can include aqueous and nonaqueous, isotonic sterile injection solutions, which can contain antioxidants, buffers, bacteriostats, and solutes that render the formulation isotonic with the blood of the intended recipient, and aqueous and nonaqueous sterile suspensions that can include suspending agents, solubilizers, thickening agents, stabilizers, and preservatives. The formulations can be presented in unit dose or multi-dose sealed containers, such as ampules and vials, and can be stored in a freeze-dried (lyophilized) condition requiring only the addition of the sterile liquid carrier, for example, water, for injections, immediately prior to use. Extemporaneously injectable solutions and suspensions can be prepared from sterile powders, granules, and tablets, as described herein.

A formulation suitable for oral administration can consist of liquid solutions, such as an effective amount of the compound dissolved in diluents, such as water, saline, or fruit juice; capsules, sachets or tablets, each containing a predetermined amount of the active ingredient, as solid or granules; solutions or suspensions in an aqueous liquid; and oil-in-water emulsions or water-in-oil emulsions. Tablet forms can include one or more of lactose, mannitol, corn starch, potato starch, microcrystalline cellulose, acacia, gelatin, colloidal silicon dioxide, croscarmellose sodium, talc, magnesium stearate, stearic acid, and other excipients, colorants, diluents, buffering agents, moistening agents, preservatives, flavoring agents, and pharmacologically compatible carriers.

Similarly, a formulation suitable for oral administration can include lozenge forms, which can comprise the active ingredient in a flavor, usually sucrose and acacia or tragacanth; pastilles comprising the active ingredient in an inert base, such as gelatin and glycerin, or sucrose and acacia; and mouthwashes comprising the active ingredient in a suitable liquid carrier; as well as creams, emulsions, gels, and the like containing, in addition to the active ingredient, such carriers as are known in the art.

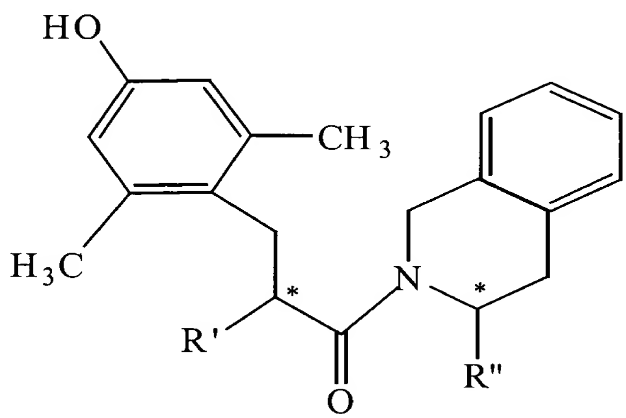
An aerosol formulation suitable for administration via inhalation also can be made. The aerosol formulation can be placed into a pressurized acceptable propellant, such as dichlorodifluoromethane, propane, nitrogen, and the like.

A formulation suitable for topical application can be in the form of creams, ointments, or lotions.

A formulation for rectal administration can be presented as a suppository with a suitable base comprising, for example, cocoa butter or a salicylate. A formulation suitable for vaginal administration can be presented as a pessary, tampon, cream, gel, paste, foam, or spray formula containing, in addition to the active ingredient, such carriers as are known in the art to be appropriate.

Any of the above compositions can further comprise one or more other active agents. Alternatively, any of the above compositions can be administered, by the same or different route, in combination with another composition comprising one or more other active agents, either simultaneously or sequentially in either order sufficiently close in time to realize the benefit of such co-administration.

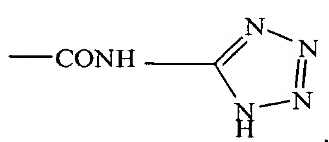
In view of the above, the present invention provides a method of treating a mammal in need of an antagonist of a δ-opioid receptor. The method comprises administering at least one compound of formula:



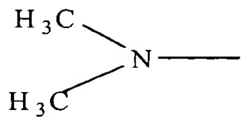
wherein (i) R' is H_2N— and R'' is —CH_2COOH ,

(ii) R' is H_2N— and

R'' is

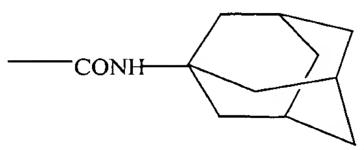


(iii) R' is

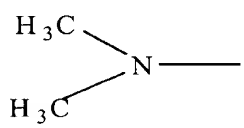


and

R" is

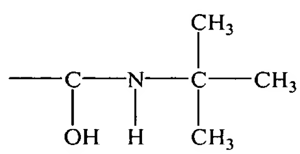


(iv) R' is



and

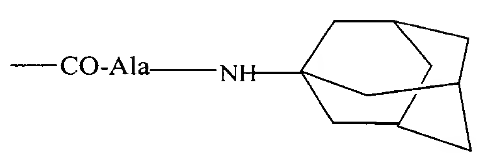
R" is



,

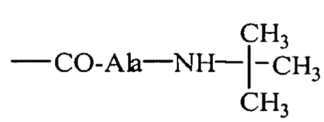
(v) R' is H_2N— and

R" is

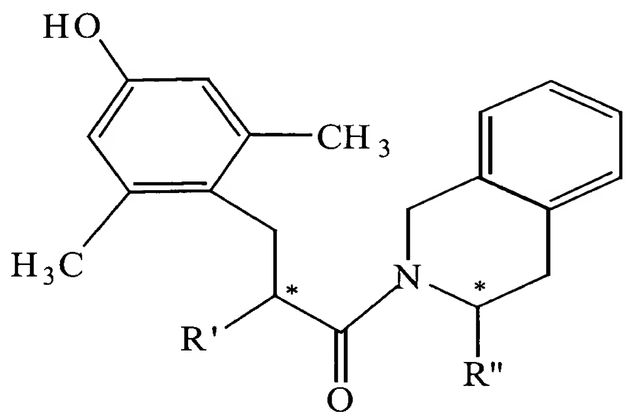


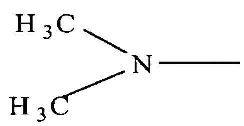
or (vi) R' is H_2N— and

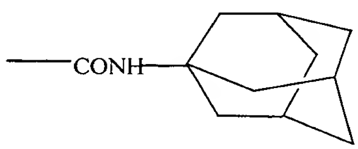
R" is



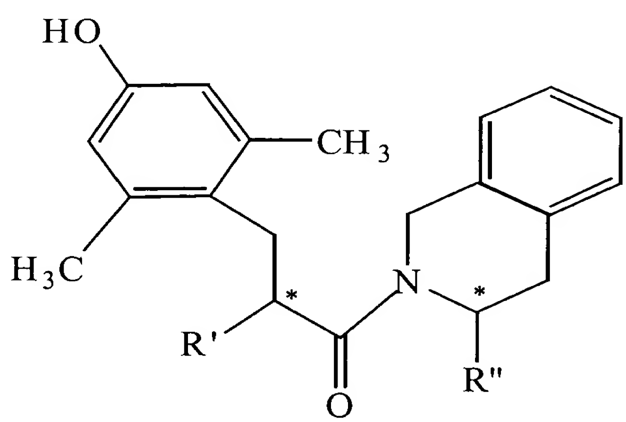
in an amount that antagonizes a δ-opioid receptor in said mammal. In the context of the above method, if the mammal is also in need of an agonist of a μ-opioid receptor, the compound of formula:



is the compound wherein R' is  and R" is

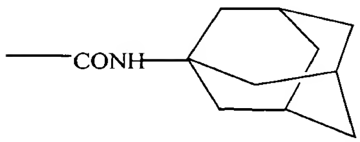
 and the compound is administered in an amount that also agonizes a μ-opioid receptor in said mammal. Such a method and compounds are useful to induce analgesia.

The present invention further may provide a method of treating a mammal in need of an agonist of a δ-opioid receptor. The method may comprise administering at least one compound of formula:



wherein (i) R' is H_2N— and

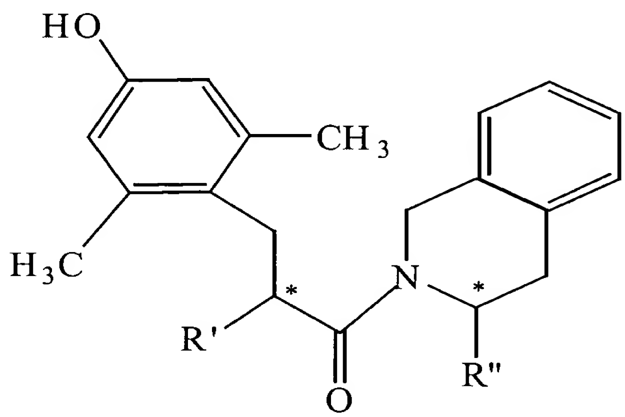
R" is



or

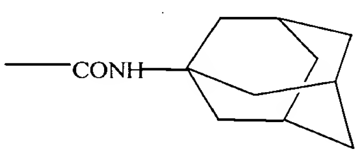
(ii) R' is H_2N— and R" is —CO-Ala—$NHCH_3$ in an amount that agonizes a δ-opioid receptor in said mammal.

Also, a method of treating a mammal in need of an agonist of a μ-opioid receptor may be provided. The method may comprise administering at least one compound of formula:



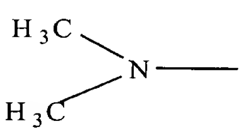
wherein (i) R' is H_2N— and

R" is



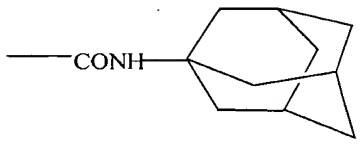
,

(ii) R' is



and

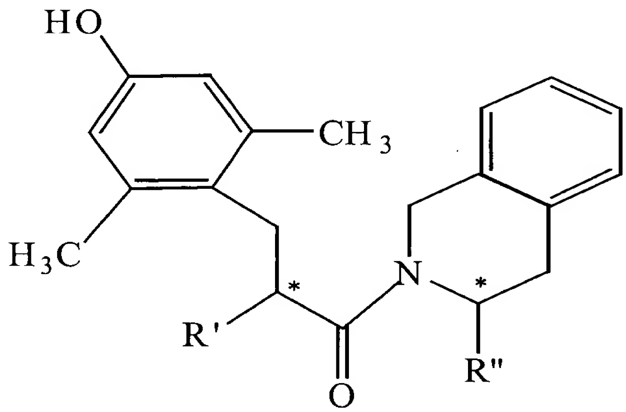
R" is



, or

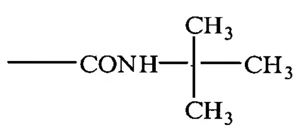
(iii) R' is H_2N— and R'' is —CO-Ala—$NHCH_3$ in an amount that agonizes a μ-opioid receptor in said mammal.

Still further provided is a method of inhibiting the binding of an opioid receptor-binding compound with a P glycoprotein in a mammal. The method comprises administering at least one compound of formula:



wherein (i) R' is H_2N— and

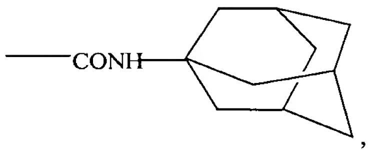
R'' is



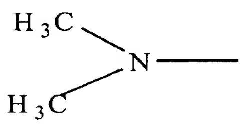
,

(ii) R' is H_2N— and

R'' is

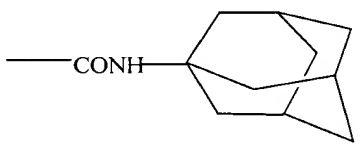


(iii) R' is



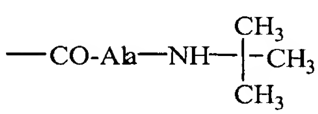
and

R" is



(iv) R' is H_2N— and

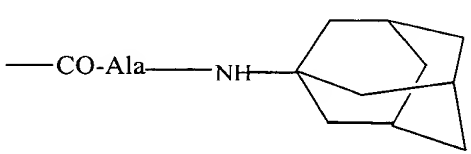
R" is



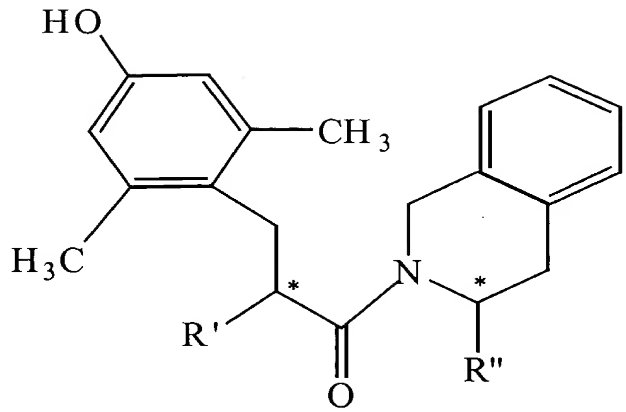
, or

(v) R' is H_2N— and

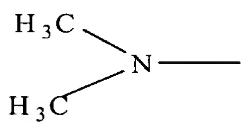
R" is



in an amount effect to inhibit the binding of an opioid receptor-binding compound with a P glycoprotein in a mammal. Preferably the P glycoprotein is P-gp1 (hMDR-1). Preferably the compound of formula:

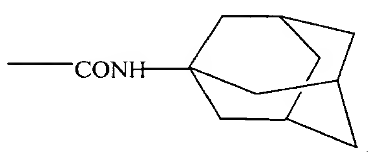


is the compound wherein R' is



and

R'' is



Such a method is useful to sensitize a mammal to verapamil or asimadoline (EMD 61753), thereby reducing its dosage and decreasing any adverse side effects. Such a method is also useful to sensitive cancer cells to a chemotherapeutic agent in the treatment of cancer, such as solid tumors, e.g., breast cancer, and various lymphomas, such as those that are resistant to currently available chemotherapeutic agents and the like. The present invention compounds are advantageous as sensitizing agents because they are not toxic.

The dose administered to a mammal, particularly a human, in the context of the present invention should be sufficient to effect a therapeutic response in the individual over a reasonable time frame. The dose will be determined by the potency of the particular compound employed for treatment, the severity of any condition to be treated, as well as the body weight and age of the individual. The size of the dose also will be determined by the existence of any adverse side effects that may accompany the use of the particular compound employed. It is always desirable, whenever possible, to keep adverse side effects to a minimum.

The dosage can be in unit dosage form, such as a tablet or capsule. The term "unit dosage form" as used herein refers to physically discrete units suitable as unitary dosages for human and animal subjects, each unit containing a predetermined quantity of a compound, alone or in combination with other active agents, calculated in an amount sufficient to produce the desired effect in association with a pharmaceutically acceptable diluent, carrier, or vehicle. The specifications for the unit dosage forms of the present invention depend on the particular embodiment employed and the effect to be achieved, as well as the pharmacodynamics associated with each compound in the host. The dose administered should be an effective amount, i.e., an amount effective to antagonize or agonize a δ-opioid receptor or a μ-opioid receptor as desired.

Since the "effective amount" is used as the preferred endpoint for dosing, the actual dose and schedule can vary, depending on interindividual differences in pharmacokinetics, drug distribution, and metabolism. The "effective amount" can be defined, for example, as the blood or tissue level desired in the patient that corresponds to a concentration of one or more compounds according to the invention. The "effective amount" for a given compound of the present invention also can vary when the composition of the present invention comprises another active agent or is used in combination with another composition comprising another active agent.

One of ordinary skill in the art can easily determine the appropriate dose, schedule, and method of administration for the exact formulation of the composition being used, in order to achieve the desired "effective amount" in the individual patient. One skilled in the art also can readily determine and use an appropriate indicator of the "effective amount" of the compound of the present invention by pharmacological end-point analysis.

Further, with respect to determining the effective amount in a patient, suitable animal models are available and have been widely implemented for evaluating the *in vivo* efficacy of such compounds. These models include the tail flick test (see, e.g., U.S. Patent No. 5,780,589). *In vitro* models are also available, examples of which are set forth in the Examples herein.

Generally, an amount of a present inventive compound up to about 50 mg/kg body weight, preferably from about 10 mg/kg body weight to about 50 mg/kg body weight is preferred, especially from about 10 mg/kg body weight to about 20 mg/kg body weight. In certain applications, multiple daily doses are preferred. Moreover, the number of doses will vary depending on the means of delivery and the particular compound administered.

EXAMPLES

The following examples serve to illustrate further the present invention and are not intended to limit its scope in any way.

Nomenclature as established by the IUPAC-IUB Commission on Biochemical Nomenclature (*J. Biol. Chem.* 260: 1442 (1985)) will be used herein. In addition, the following symbols and abbreviations will be used:

AD: 1-adamantyl amide

BBB: blood-brain barrier

Boc: *tert*-butyloxycarbonyl

INS B1 ~~DAGO: H-Tyr-D-Ala-Gly-*N*-MePhe-Gly-ol (μ agonist)~~

INS B2 ~~DAMME: H-Tyr-D-Ala-Gly-*N*-MePhe-Met(O)-ol (μ agonist)~~

DCC: *N,N'*-dicyclohexylcarbodiimide

INS B3 ~~Deltorphin A: H-Tyr-D-Ala-Phe-Glu-Val-Val-Gly-NH_2 (δ_1 agonist)~~

INS B4 ~~Deltorphin B: H-Tyr-D-Ala-Phe-Glu-Val-Val-Gly-NH_2 (δ_2 agonist)~~

INS B5 ~~DER: dermorphin or H-Tyr-D-Ala-Phe-Gly-Tyr-Pro-Ser-NH_2 (μ agonist)~~

DMF: *N,N*-dimethylformamide

DMSO: dimethylsulfoxide

Dmt or D: 2',6'-dimethyl-L-tyrosine

INS B6 ~~DPD: H-Dmt-D-Phe-Gly-Val-Val-NH_2 (μ antagonist)~~

B7 ~~DPDPE: H-Tyr-[D-Pen-Gly-Phe-D-Pen] (δ_1 agonist)~~

DSB: H-Dmt-Sar-Bid (δ/μ agonist)

DTA: H-Dmt-Tic-Ala-NH_2 (δ antagonist)

DtMe: H-Dmt-Tic-$NHCH_3$ (δ antagonist)

Et: ethyl

Et_2O: diethyl ether

EtPt: petroleum ether

GPI: guinea pig ileum (μ receptor bioassay)

H-αDmt-OH: 2-methylamino-3-(2',6'-dimethyl-4-hydroxyphenyl)-propionic acid

H-βTic-OH: 1,2,3,4-tetrahydroisoquinoline-3-yl acetic acid

hMDR-1: human multidrug resistance glycoprotein (Pgp)-1

HOBt: 1-hydroxybenzotriazole

HPLC: high performance liquid chromatography

K_e: the antilog of pA_2 in molar concentration

$LiAlH_4$: lithium aluminum hydride

Me: methyl

MeDTOH: *N,N*$(Me)_2$-Dmt-Tic-OH (δ antagonist)

MeOH: methanol

MVD: mouse vas deferens (δ specific bioassay)

$NaBH_3CN$: sodium cyanoborohydride

NAL: naltrindole

NH-tBut: *tert*-butyl amine

NMM: *N*-methyl morpholine

OMe: methyl ester

PA_2: negative log of the molar concentration required to double the agonist concentration to achieve the original response

PipDTOH: [des-NH_2-α-piperidine-1-yl]Dmt-Tic-OH (δ antagonist)

*t*Bu: *tert*-butyl

TEA: triethylamine

TFA: trifluoroacetic acid

Tic or T: 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid

TIP(P): H-Tyr-Tic-Phe-(Phe)-OH

TLC: thin layer chromatography

ins B8 ~~[Trp^4,Tyr^5]DER: H-Tyr-D-Ala-Phe-Trp-Tyr-NH_2 (μ agonist)~~

ins B9 ~~[Trp^4,Lys-OH^7]DER: H-Tyr-D-Ala-Phe-Trp-Tyr-Pro-Lys-OH (μ agonist)~~

WSC: 1-ethyl-3-[3'-dimethyl-aminopropyl]carbodiimide hydrochloride

Z: benzyloxycarbonyl

Verapamil, colchicine and sodium pyruvate were obtained from Sigma Chemical Co. (St. Louis, MO). DPDPE and DAGO were obtained from Bachem (Torrance, CA). Boc-Tic-OH was obtained from Bachem Feinchemikalien AG (Bubendorf, Switzerland). Naloxone, naltrindole and BNTX were obtained from Research Biochemicals International (Natick, MA). Vybrant MDR assay is a product of Molecular Probes (Eugene, OR).

[^{3}H]DPDPE was from NEN-DuPont (Bilirica, MA) and [^{3}H]DAGO (60 Ci/mmol) was from Amersham (Arlington Heights, IL).

H-L-Dmt-OH was synthesized as previously described (Dygos et al., *Synthesis* 8: 741-743 (1992)). All peptides were prepared by standard solution methods (Lazarus et al., *Biochem. Biophys. Res. Commun.* 178: 110-115 (1991); Sagan et al., *Biochem. Biophys. Res. Commun.* 187: 1203-1210 (1992)). Dipeptides were obtained by condensation of Boc-L-Dmt-OH with Tic derivatives (H-Tic-OtBu, H-Tic-NHMe, H-Tic-NH-1-adamantane and H-βTic-OMe) or by condensation of Boc-Dmt-Tic-OH with *tert*-butyl amine or 5-aminotetrazolyl via DCC/HOBt. Tripeptides were obtained by condensation of Boc-L-Dmt-Tic-OH with Ala derivatives (H-Ala-NHMe, H-Ala-NH-1-adamantane, H-Ala-NH-tBu and H-Ala-OMe) via DCC/HOBt. Final products were obtained, when necessary, by ester function hydrolysis with 1 N NaOH and removal of the Boc protecting group in TFA. *N*-alkylated di- and tripeptide derivatives were obtained by reductive alkylation of the corresponding deprotected linear peptides with aldehydes (formaldehyde, glutaraldehyde, succinaldehyde) and $NaBH_3CN$ in acetonitrile (Borch et al., *J. Org. Chem.* 37: 1673-1674 (1972)). HCl•H-βH-Tic-OMe was prepared by reduction of H-Tic-OMe with $LiAlH_4$ to the corresponding 3-hydroxymethyl-Tic that was transformed first in 3-bromomethyl-Tic and then in 3-cyano-Tic. Treatment of the cyano group with HCl-methanol gave the final product (Crabb et al., *J. Chem. Soc. Perkins Trans. II*: 370-378 (1977)). α-H(R,S)-Dmt-Tic-OH was obtained by condensation of (R,S)-2-cyano-3-(4-hydroxy-2,6-dimethylphenyl)-propanoic acid with H-Tic-OtBu via DCC/HOBt. In turn, (R,S)-2-cyano-3-(4-hydroxy-2,6-dimethylphenyl)-propanoic acid was prepared from ethyl cyanoacetate and O-carbethoxy-3,5-dimethyl-4-chloromethylphenol (Abrash et al., *Biochemistry* 2: 947-952 (1972)).

Preparative reversed-phase HPLC was conducted with a Waters Delta Prep 3000 Å (30 x 3 cm; 15 μm) column. Peptides were eluted with a gradient of 0-60% B in 25 min at a flow rate of 50 ml/min using the following mobile phases: solvent A (10% acetonitrile in 0.1% TFA, v/v) and solvent B(60% acetonitrile in 0.1% TFA, v/v). Analytical HPLC analyses were carried out with a Bruker Liquid Chromatographer LC21-C instrument using a Vydac 218 TP 5415 C18 column (250 x 4.6 mm, 5 μm particle size) and equipped with a Bruker LC 313 UV variable wavelength detector. Recording and quantification were accomplished with a chromatographic data processor coupled to an Epson computer system (QX-10). Analytical determinations were determined using HPLC conditions in the above

solvent systems programmed at a flow rate of 1 ml/min in a linear gradient from 0-100% B in 25 min. All analogues showed less than 1% impurities when monitored at 220 nm.

TLC was performed on precoated plates of silica gel F254 (Merck, Darmstadt, Germany) using the following solvent systems: (a) 1-butanol/AcOH/H_2O (3:1:1, v/v/v) and (B) CH_2Cl_2/toluene/methanol (17:1:2, v/v/v). Ninhydrin (1%, Merck), fluorescamine (Hoffman-LaRoche), and chlorine reagents were used as sprays. Open column chromatography (2 x 70 cm, 0.7-1 g material) was used on silica gel 60 (70-230 mesh, Merck) using the same eluent systems.

Melting points were determined on a Kofler apparatus and are uncorrected. Optical rotations were determined at 10 mg/ml in methanol with a Perkin-Elmer 241 polarimeter with a 10 cm water-jacketed cell. All ^{1}H-NMR spectra were recorded on a Bruker 200 MHZ spectrometer. Matrix-assisted laser desorption ionization time of flight (MALDI-TOF) mass spectrometry analyses of peptides were conducted using a Hewlett Packard G 2025 A LD-TOF system. The samples were analyzed in the linear mode with 28 kV accelerating voltage, mixing them with a saturated solution of α-cyano-4-hydroycinnamic acid matrix.

Example 1

This example describes the synthesis of Boc-Dmt-Tic-OMe.

HOBt (0.22 g, 1.42 mmol) and DCC (0.29 g, 1.42 mmol) were added to a solution of Boc-Dmt-OH (0.4 g, 1.29 mmol) and H-Tic-OMe (0.25 g, 1.29 mmol, Hayashi et al., *Chem. Pharm. Bull.* 31: 312-314 (1983)). The reaction was stirred for 3 hr at 0°C and for 24 hr at room temperature (RT). After evaporation of DMF, the residue was solubilized in EtOAc and washed with citric acid (10%), $NaHCO_3$ (5%) and brine. The organic phase was dried and evaporated to dryness. The residue was crystallized from Et_2O/PtEt (1:1, v/v): yield 0.52 g (83%); R_f (B) 0.81; HPLC K' = 7.91; mp 135-137 °C; $[\alpha]^{20}D$ -13.2; MH^+ 483; ^{1}H-NMR (DMSO) δ = 1.37-1.46 (d, 9H), 2.16 (s,6H), 2.96-3.01 (m, 2H), 3.08-3.13 (m, 2H), 3.46-3.56 (m, 1H), 3.72 (s, 3H), 4.34-4.88 (m, 3H), 6.46 (s, 2H), 7.18-7.21 (m, 4H), 8.32 (bs, 1H).

Example 2

This example describes the synthesis of Boc-Dmt-Tic-$NHNH_2$.

$NH_2NH_2 \bullet H_2O$ (1 ml) was added to a solution of Boc-Dmt-Tic-OMe (0.26 g, 0.54 mmol) in MeOH (10 ml). The reaction mixture was stirred for 24 hr at RT. After evaporation of the solvent, the residue was crystallized from Et_2O/PtEt (1:1, v/v): yield 0.25 g (94%); R_f(B) 0.75; HPLC K' = 6.83; mp 154-156 °C; $[\alpha]^{20}D$ -14.5; MH^+ 483; ^{1}H-NMR (DMSO) δ = 1.35-1.44 (d, 9H), 2.16 (s, 6H), 3.07-3.40 (s, 4H), 3.79 (m, 1H), 4.29-4.78 (m, 5H), 6.34 (m, 2H), 6.95 (bs, 1H), 7.14 (3, 4H), 8.22 (bs, 1H).

Example 3

This example describes the synthesis of TFA-H-Dmt-Tic-$NHNH_2$ (**compound 6** in Table II).

Boc-Dmt-Tic-$NHNH_2$ (0.2 g, 0.41 mmol) was treated with TFA (1 ml) for 0.5 hr at RT. ET_2O/PtEt (1:1, v/v) was added to the solution until the product precipitated: yield 0.18 g (91%); R_f (A) 0.69; HPLC K' = 2.52; mp 143-145 °C; $[\alpha]^{20}D$ +13.5; MH^+ 382. Anal. ($C_{12}H_{26}N_4O_3 \bullet$TFA) C,H,N (C 65.95, H 6.88, N 14.65).

Example 4

This example describes the synthesis of Z-Tic-NHMe.

NMM (0.35 ml, 3.21 mmol), HOBt (0.54 g, 3.53 mmol) and DCC (0.73 g, 3.53 mmol) were added to a solution of Z-Tic-OH (1 g, 3.21 mmol, Abrash (1972), *supra*) and $HCl \bullet H_2NMe$ (0.22 g, 3.21 mmol) in DMF (10 ml) at 0 °C. The reaction mixture was stirred for 3 hr at 0 °C and 24 hr at RT. After evaporation of DMF, the residue was solubilized in EtOAc and washed with citric acid (10%), $NaHCO_3$ (5%) and brine. The organic phase was dried and evaporated to dryness. The residue was crystallized from Et_2O/PtEt (1:1, v/v): yield 0.90 g (87%); R_f (B) 0.97; HPLC K' = 8.56; mp 137-139 °C; $[\alpha]^{20}D$ +13.5; MH^+ 325; ^{1}H-NMR (DMSO) δ = 2.44 - 2.46 (d, 3H), 3.12-3.24 (m, 2H), 4.36-4.46 (m, 3H), 5.17 (s, 2H), 7.18-7.21 (m, 5H), 7.36-7.39 (m, 4H), 7.74-7.77 (m, 1H).

Example 5

This example describes the synthesis of H-Tic-NHMe.

C/Pd (5%, 0.05 g) was added to a solution of Z-Tic-NHMe (0.90 g, 2.78 mmol) in MeOH (30 ml) and H_2 was bubbled for 1 hr at RT. After filtration, the solution was evaporated to dryness. The residue was crystallized from Et_2O/PtEt (1:1, v/v): yield 0.49 g (92%); R_f (B) 0.38; HPLC K' = 5.03; mp 123-125 °C; $[\alpha]^{20}D$ +18.7; MH^+ 191.

Example 6

This example describes the synthesis of Boc-Dmt-Tic-NHMe.

Boc-Dmt-OH was condensed with H-Tic-NHMe via DCC/HOBt as described in Example 1 in order to obtain Boc-Dmt-Tic-NHMe: yield 0.13 g (85%); R_f (B) 0.73; HPLC K' = 6.34; mp 142-146 °C; $[\alpha]^{20}D$ -15.3; MH^+ 482; ^{1}H-NMR (DMSO) δ = 1.35-1.44 (d, 9H), 2.16 (s, 6H), 2.44-2.46 (d, 3H), 3.05-3.41 (m, 4H), 3.79 (m, 1H), 4.29-4.78 (m, 3H), 6.34 (s, 2H), 6.95 (bs, 1H), 7.14 (s, 4H), 8.22 (bs, 1H).

Example 7

This example describes the synthesis of TFA•H-Dmt-Tic-NHMe (**compound 7** in Table II).

Boc-Dmt-Tic-NHMe was treated with TFA as reported in Example 3: yield 0.12 g (93%); R_f (A) 0.69; HPLC K' = 3.10; mp 152-154 °C; $[\alpha]^{20}D$ = 22.0; MH^+ 382. Anal. ($C_{22}H_{27}N_3O_3$•TFA) C, H, N (C 69.27, H 7.13, N 11.02).

Example 8

This example describes the synthesis of TFA-*N*,*N*-$(Me)_2$-Dmt-Tic-NHMe (**compound 13** in Table II).

This compound was obtained by exhaustive methylation of TFA•H-Dmt-Tic-NHMe as described in Example 30: yield 0.12 g (96%); R_f (A) 0.71; HPLC K' = 3.19; mp 156-158 °C; $[\alpha]^{20}D$ -19.3; MH^+ 410. Anal. ($C_{24}H_{31}N_3O_3$•TFA) C, H, N (C 70.39, H 7.63, N 10.26).

Example 9

This example describes the synthesis of TFA•H-Dmt-Tic-OMe (**compound 8** in Table II).

Boc-Dmt-Tic-OMe was treated with TFA as described in Example 3: yield 0.25 g (92%); R_f (A) 0.81; HPLC K' = 3.83; mp 118-120 °C; $[\alpha]^{20}D$ -24.0; MH^+ 382. Anal. ($C_{22}H_{26}N_2O_4$•TFA) C, H, N (C 69.09, H 6.85, N 7.32).

Example 10

This example describes the synthesis of Boc-Dmt-βHTic-OMe.

This compound was obtained by condensation of Boc-Dmt-OH with HCl•βHTic-OMe (Crabb et al., *J. Chem. Soc. Perkins Trans. II*: 370-378 (1977)) [R_f (B) 0.38, HPLC K' = 2.31, mp 153-155 °C, $[\alpha]^{20}D$ -37.5; MH^+ 206] via DCC/HOBt as reported for Boc-Dmt-Tic-OMe: yield 0.42 g (97%); R_f (B) 0.93; HPLC K' = 9.27; mp 94-96 °C; $[\alpha]^{20}D$ +33.9; MH^+ 497; ^{1}H-NMR (DMSO) δ = 1.82 (s, 9H), 2.35 (s, 6H), 2.80-3.50 (m, 6H), 3.70 (s, 3H), 3.90 (m, 1H), 4.30 (m, 2H), 4.40 (dd, 1H), 6.43 (s, 2H), 7.20 (m, 6H), 9.50 (bs, 1H).

Example 11

This example describes the synthesis of Boc-Dmt-βHTic-OH.

Sodium hydroxide (1N, 1.34 ml) was added to a solution of Boc-Dmt-βTic-OMe (0.42 g, 0.90 mmol) in MeOH (10 ml). The reaction mixture was stirred for 24 hr at RT. After evaporation of the solvent, the residue was solubilized in EtOAc and washed with citric acid (10%) and brine. The organic phase was dried and evaporated to dryness. The residue was crystallized from Et_2O: yield 0.42 g (98%); R_f (B) 0.35; HPLC K' = 7.54; mp 117-119 °C; $[\alpha]^{20}D$ +36.2; MH^+ 483.

Example 12

This example describes the synthesis of TFA•H-Dmt-βHTic-OH (**compound 1** in Table II).

Boc-Dmt-βHTic-OH was treated with TFA as described in Example 3: yield (0.26 g (68%); R_f (A) 0.82; HPLC K' = 4.9; mp 118-120 °C; $[\alpha]^{20}D$ +95.0; MH^+ 383; ^{1}H-NMR

(DMSO) δ = 2.2 (s, 6H), 2.80-3.0 (m, 4H), 3.4 (s, 2H), 4.1 (m, 1H), 4.3 (m, 2H), 4.5 (m, 1H), 6.43 (s, 2H), 7.20 (m, 4H), 9.2 (bs, 3H). ($C_{22}H_{26}N_2O_4$•TFA) C, H, N (C 69.09, H 6.85, N 7.32).

Example 13

This example describes the synthesis of Boc-Dmt-Tic-OH.

Sodium hydroxide (1 N, 1.3 ml) was added to a solution of Boc-Dmt-Tic-OMe (0.52 g, 1.08 mmol) in MeOH (10 ml). The reaction mixture was stirred for 24 hr at RT and treated as described in Example 11: yield 0.45 g (89%); R_f (B) 0.36; HPLC K' = 6.71; mp 147-149 °C; $[\alpha]^{20}$D -15.6; MH^+ 469.

Example 14

This example describes the synthesis of Boc-Dmt-Tic-NH-tetrazole-5-yl.

HOBt (0.14 g, 0.94 mmol) and DCC (0.19 g, 0.94 mmol) were added to a solution of Boc-Dmt-Tic-OH (0.4 g, 0.85 mmol) and 5 aminotetrazole monohydrate (0.1 g, 0.85 mmol) in DMF (10 ml) at 0 °C. The reaction mixture was stirred for 3 hr at 0 °C and 24 hr at RT. After evaporation of the DMF, it was treated as described in Example 11; however, the residue was crystallized from Et_2O/PtEt (1:1, v/v): yield 0.19 g (42%); R_f (B) 0.75; HPLC K' = 7.18; mp 146-148 °C; $[\alpha]^{20}$D -23.1; MH^+ 537; ^{1}H-NMR (DMSO) δ = 1.36-1.45 (d, 9H), 2.16 (s, 6H), 2.96-3.0 (m, 2H), 3.08-3.13 (m, 2H), 3.46-3.56 (m, 1H), 4.34-4.88 (m, 3H), 6.46 (s, 2H), 7.18-7.21 (m, 4H), 8.32 (bs, 1H), 8.45 (bs, 1H), 14.39 (bs, 1H).

Example 15

This example describes the synthesis of TFA•H-Dmt-Tic-NH-tetrazole-5-yl (**compound 10** in Table II).

Boc-Dmt-Tic-NH-tetrazole-5-yl was treated with TFA as described in Example 3: yield 0.17 g (87%); R_f(A) 0.79; HPLC K' = 3.75; mp 131-133 °C; $[\alpha]^{20}$D -18.3; MH^+ 436; ^{1}H-NMR (DMSO) δ = 2.18 (s, 6H), 2.81-3.03 (m, 2H), 3.12-3.18 (m, 2H), 3.66-3.76 (m, 1H), 4.38-4.52 (m, 3H), 6.45 (s, 2H), 7.21-7.25 (m, 4H), 8.27 (bs, 3H), 8.9 (bs, 3H), 14.39 (bs, 1H). Anal. ($C_{22}H_{25}N_7O_3$•TFA) C, H, N (C 69.68, H 5.79, N 22.51).

Example 16

This example describes the synthesis of Boc-Dmt-Tic-Ala-OMe.

NMM (0.05 ml, 0.42 mmol), HOBt (0.07 g, 0.46 mmol) and DCC (0.09 g, 0.46 mmol) were added to a solution of Boc-Dmt-Tic-OH (0.2 g, 0.42 mmol) and HCl•H-Ala-OMe (0.06 g, 0.42 mmol) in DMF (10 ml) at 0 °C. the reaction was stirred for 3 hr at 0 °C and 24 hr at RT. After evaporation of DMF, the residue was treated as described in Example 14: yield 0.2 g (85%); R_f (B) 0.77; HPLC K' = 7.68; mp 124-126 °C; $[\alpha]^{20}D$ +40.1; MH^+ 555; ^{1}H-NMR (DMSO) δ = 1.37-1.46 (m, 12H), 2.16 (s, 6H), 2.96-3.01 (m, 2H), 3.08-3.13 (m, 2H), 3.46-3.56 (m, 1H), 3.73 (s, 3H), 4.03-4.07 (q, 1H), 4.34-4.88 (m, 3H), 6.46 (s, 2H), 7.18-7.21 (m, 4H), 8.32 (bs, 1H), 8.51 (bs, 1H).

Example 17

This example describes the synthesis of TFA•H-Dmt-Tic-Ala-OMe (**compound 19** in Table II). Boc-Dmt-Tic-Ala-OMe was treated with TFA as described in Example 3: yield 0.19 g (92%); R_f (A) 0.64; HPLC K' = 3.72; mp 130-132 °C; $[\alpha]^{20}D$ +90.1; MH^+ 454. Anal. ($C_{25}H_{31}N_3O_3$•TFA) C, H, N (C 66.21, H 6.89, N 9.26).

Example 18

This example describes the synthesis of Boc-Tic-NH-1-adamantane.

NMM (0.20 ml, 1.8 mmol), HOBt (0.30 g, 1.98 mmol) and DCC (0.41 g, 1.98 mmol) were added to a solution of Boc-Tic-OH (0.5 g, 1.8 mmol) and 1-aminoadamantane hydrochloride (0.34 g, 1.8 mmol) in DMF (10 ml) at 0 °C. The reaction was stirred for 3 hr at 0 °C and 24 hr at RT. After evaporation of DMF, the residue was treated as described in Example 14: yield 0.6 g (81%); R_f(B) 0.89; HPLC K' = 9.68; mp 127-129 °C; $[\alpha]^{20}D$ +15.3; MH^+ 412; ^{1}H-NMR (DMSO) δ = 1.39.-1.46 (d, 9H), 1.65 (s, 6H), 1.93-2.07 (m, 9H), 3.08-3.13 (m, 2H), 4.34-4.88 (m, 3H), 7.17-7.20 (m, 4H), 8.08 (bs, 1H).

Example 19

This example describes the synthesis of TFA•H-Tic-NH-1-adamantane.

Boc-Tic-NH-1-adamantane (0.6 g; 1.46 mmol) was treated with TFA (2 ml) for 0.5 hr at RT. Et_2O/EtPt (1:1, v/v) was added to the solution until the product precipitated: yield 0.57 g (92%); R_f (A) 0.73; HPLC K' = 5.80; mp 157-159 °C; $[\alpha]^{20}D$ +18.4; MH^+ 311.

Example 20

This example describes the synthesis of Boc-Dmt-Tic-NH-1-adamantane.

This compound was obtained by condensation of Boc-Dmt-OH with TFA-H-Tic-NH-1-adamantane via DCC/HOBt as described in Example 1: yield 0.16 g (85%); R_f (B) 0.93; HPLC K' = 9.28; mp 142-144 °C; $[\alpha]^{20}D$ +28.1; MH^+ 603; ^{1}H-NMR (DMSO) δ = 1.38-1.45 (d, 9H), 1.64 (s, 6H), 1.93-2.08 (m, 9H), 2.17 (s, 6H), 2.96-3.01 (m, 2H), 3.08-3.13 (m, 2H), 3.47-3.54 (m, 1H), 4.34-4.88 (m, 3H), 6.46 (s, 2H), 7.17-7.20 (m, 4H), 8.27 (bs, 1H), 8.47 (bs, 1H).

Example 21

This example describes the synthesis of TFA•H-Dmt-Tic-NH-1-adamantane (**compound 11** in Table II).

Boc-Dmt-Tic-NH-1-adamantane was treated with TFA as described in Example 3: yield 0.15 g (94%; R_f (A) 0.64; HPLC K' = 6.98; mp 180-182 °C; $[\alpha]^{20}D$ -2.7; MH^+ 502. Anal. ($C_{31}H_{39}N_3O_3$•TFA) C, H, N (C 74.22, H 7.84, N 8.38).

Example 22

This example describes the synthesis of Boc-D-Tic-NH-1-adamantane.

This compound was obtained by condensation of Boc-D-Tic-OH with 1-amino adamantane hydrochloride as described in Example 18: yield 0.6 g (81%); R_f (B) 0.89; HPLC K' = 9.68; mp 127-129 °C; $[\alpha]^{20}D$ -15.3; MH^+ 412; ^{1}H-NMR (DMSO) δ = 1.39-1.46 (d, 9H), 1.65 (s, 6H), 1.93-2.07 (m, 9H), 3.08-3.13 (m, 2H), 4.34-4.88 (m, 3H), 7.17-7.20 (m, 4H), 8.08 (bs, 1H).

Example 23

This example describes the synthesis of TFA•H-D-Tic-NH-1-adamantane.

Boc-D-Tic-NH-1-adamantane was treated with TFA as reported for TFA•H-Tic-NH-1-adamantane: yield 0.57 g (92%); R_f (A) 0.73; HPLC K' = 5.80; mp 157-159 °C; $[\alpha]^{20}D$ -18.4; MH^+ 311.

Example 24

This example describes the synthesis of Boc-Dmt-D-Tic-NH-1-adamantane.

This compound was obtained by condensation of Boc-Dmt-OH with TFA-H-D-Tic-NH-1-adamantane via DCC/HOBt as described in Example 1: yield 0.16 g (85%); R_f (B) 0.87; HPLC K' = 9.54; mp 135-137 °C; $[\alpha]^{20}D$ +14.2; MH^+ 603; ^1H-NMR (DMSO) δ = 1.39-1.46 (d, 9H), 1.63 (s, 6H), 1.94-2.09 (m, 9H), 2.16 (s, 6H), 2.97-3.03 (m, 2H), 3.05-3.14 (m, 2H), 3,49-3.56 (m, 1H), 4.41-4.91 (m, 3H), 6.41 (s, 2H), 7.18-7.20 (m, 4H), 8.31 (bs, 1H), 8.43 (bs, 1H).

Example 25

This example describes the synthesis of TFA•H-Dmt-D-Tic-NH-1-adamantane (**compound 12** in Table II).

Boc-Dmt-D-Tic-NH-1-adamantane was treated with TFA as described in Example 3: yield 0.15 g (94%); R_f (A) 0.58; HPLC K' = 7.24; mp 164-166 °C; $[\alpha]^{20}D$ +27.5; MH^+ 502. Anal. ($C_{31}H_{39}N_3O_3$•TFA) C, H, N (C 74.22, H 7.84, N 8.38).

Example 26

This example describes the synthesis of Boc-Ala-NH-1-adamantane.

NMM (0.20 ml, 1.8 mmol), HOBt (0.30 g, 1.98 mmol) and DCC (0.41 g, 1.98 mmol) were added to a solution of Boc-Ala-OH (0.34 g, 1.8 mmol) and 1-amino adamantane hydrochloride (0.34 g, 1.8 mmol) in DMF (10 ml) at 0 °C. The reaction was stirred for 3 hr at 0 °C and 24 hr at RT. After evaporation of DMF, the residue was treated as described in Example 14: yield 0.52 g (89%); R_f (B) 0.85; HPLC K' = 7.73; mp 107-109 °C; $[\alpha]^{20}D$ +5.9; MH^+ 324; ^1H-NMR (DMSO) δ = 1.38-1.45 (m, 12H), 1.65 (s, 6H), 1.93-2.07 (m, 9H), 4.04-4.07 (q, 1H), 8.08 (bs, 1H), 8.42 (bs, 1H).

Example 27

This example describes the synthesis of TFA•H-Ala-NH-1-adamantane.

Boc-Ala-NH-1-adamantane (0.52 g, 1.6 mmol) was treated with TFA (2 ml) for 0.5 hr at RT. Et_2O/EtPt (1:1, v/v) was added to the solution until the product precipitated: yield 0.5 g (92%); R_f (A) 0.82; HPLC K' = 4.95; mp 131-133 °C; $[\alpha]^{20}D$ +6.8; MH^+ 224.

Example 28

This example describes the synthesis of Boc-Dmt-Tic-Ala-NH-1-adamantane.

This compound was obtained by condensation of Boc-Dmt-Tic-OH with TFA•H-Ala-NH-1-adamantane via DCC/HOBt as described in Example 16: yield 0.12 g (87%); R_f (B) 0.91; HPLC K' = 9.32; mp 137-139 °C; $[\alpha]^{20}D$ +15.5; MH^+ 674; ^1H-NMR (DMSO) δ = 1.38-1.45 (d, 12H), 1.64 (s, 6H), 1.93-2.08 (m, 9H), 2.17 (s, 6H), 2.96-3.01 (m, 2H), 3.08-3.13 (m, 2H), 3.47-3.54 (m, 1H), 4.03-4.07 (m, 1H), 4.34-4.88 (m, 3H), 6.46 (s, 2H), 7.17-7.20 (m, 4H), 8.27 (bs, 1H), 8.47 (bs, 1H), 8.53 (bs, 1H).

Example 29

This example describes the synthesis of TFA•H-Dmt-Tic-Ala-NH-1-adamantane (**compound 21** in Table II).

Boc-Dmt-Tic-Ala-NH-1-adamantane was treated with TFA as described in Example 3: yield 0.11 g (92%); R_f (A) 0.65; HPLC K' = 6.91; mp 163-165°C; $[\alpha]^{20}D$ +20.1; MH^+ 574. Anal. ($C_{34}H_{44}N_4O_4$•TFA) C, H, N (C 71.3, H 7.74, N 9.78).

Example 30

This example describes the synthesis of TFA•*N,N*-$(Me)_2$-Dmt-Tic-NH-1-adamantane (**compound 15** in Table II).

NMM (0.01 ml, 0.11 mmol), 37% aqueous formaldehyde (0.07 ml, 0.83 mmol) and sodium cyanoborohydride (0.016 g, 0.25 mmol) were added to a stirred solution of TFA•H-Dmt-Tic-NH-1-adamantane (0.7 g, 0.11 mmol) in acetonitrile (10 ml). Glacial acetic acid (0.02 ml) was added over 10 min and the reaction was stirred at RT for 2 hr. The reaction mixture was evaporated *in vacuo* to give a crude product that was purified by preparative HPLC: yield 0.06 g (90%); R_f (A) 0.64; HPLC K' = 7.44; mp 118-120 °C; $[\alpha]^{20}D$ -58.01; MH^+ 530. Anal. ($C_{33}H_{43}N_3O_3$•TFA) C, H, N (C 74.82, H 8.18, N 7.93).

Example 31

This example describes the synthesis of TFA•*N,N*-$(Me)_2$-Dmt-D-Tic-Ala-NH-1-adamantane (**compound 16** in Table II).

This compound was obtained by exhaustive methylation of TFA•H-Dmt-D-Tic-NH-1-adamantane as described in Example 30: yield 0.06 g (90%); R_f (A) 0.61; HPLC K' =

7.53; mp 134-136 °C; $[\alpha]^{20}D$ +8.2; MH^+ 530. Anal. ($C_{33}H_{43}N_3O_3$•TFA) C, H, N (C 74.82, H 8.18, N 7.93).

Example 32

This example describes the synthesis of Boc-Dmt-Tic-Ala-NHMe.

This compound was obtained by condensation of Boc-Dmt-Tic-OH with HCl•H-Ala-NHMe via DCC/HOBt as described in Example 16: yield 0.25 g (84%); R_f (B) 0.78; HPLC K' = 8.90; mp 131-133 °C; $[\alpha]^{20}D$ +42.7; MH^+ 553; ^{1}H-NMR (DMSO) δ = 1.37-1.46 (m, 12H), 2.16 (s, 6H), 2.44-2.46 (d, 3H), 2.96-3.01 (m, 2H), 3.08-3.13 (m, 2H), 3.46-3.56 (m, 1H), 4.03-4.07 (q, 1H), 4.34-4.88 (m, 3H), 6.46 (s, 2H), 7.18-7.35 (m, 4H), 8.32 (bs, 1H), 8.43 (bs, 1H), 8.51 (bs, 1H).

Example 33

This example describes the synthesis of TFA•Dmt-Tic-Ala-NHMe (**compound 18** in Table II).

Boc-Dmt-Tic-Ala-NHMe was treated with TFA as described in Example 3: yield 0.24 g (91%); R_f (A) 0.58; HPLC K' = 4.29; mp 142-144 °C; $[\alpha]^{20}D$ +28.5; MH^+ 453. Anal. ($C_{25}H_{32}N_4O_4$•TFA) C, H, N (C 66.35, H 7.13, N 12.38).

Example 34

This example describes the synthesis of TFA•[des-NH_2-α-piperidine-1-yl-Dmt]-Tic-OH (**compound 3** in Table II).

NMM (0.07 ml, 0.62 mmol), 50% aqueous glutaraldehyde (0.38 ml, 2.36 mmol) and sodium cyanoborohydride (0.45 g, 0.73 mmol) were added to a stirred solution of TFA•H-Dmt-Tic-OH (0.15 g, 0.31 mmol, Matthes et al., *Nature* 383: 819-823 (1996)) in acetonitrile (10 ml). Glacial acetic acid (0.06 ml) was added over 10 min and the reaction was stirred at RT for 2 hr. The reaction mixture was evaporated *in vacuo* to give a crude product that was purified by preparative HPLC: yield 0.16 g (90%); R_f (A) 0.74; HPLC K' = 3.66; mp 205-207 °C; $[\alpha]^{20}D$ -14.2; MH^+ 437. Anal. ($C_{26}H_{32}N_2O_4$•TFA) C, H, N (C 71.53, H 7.39, N 6.42).

Example 35

This example describes the synthesis of TFA•[des-NH_2-α-pyrrolidine-1-yl-Dmt]-Tic-OH (**compound 4** in Table II) and TFA•[des-NH_2-α-pyrrole-1-yl-Dmt]-Tic-OH (**compound 5** in Table II).

These two compounds were obtained by reductive alkylation of TFA-H-Dmt-Tic-OH with succinaldehyde as described in Example 34. Analytical data for compound 4: yield 0.016 g (20%); R_f (A) 0.81; HPLC K' = 2.68; mp 168-170 °C; $[\alpha]^{20}D$ +45.2; MH^+ 423. Anal. ($C_{25}H_{30}N_2O_4$•TFA) C, H, N (C 71.07, H 7.16, N 6.63). Analytical data for compound 5: yield 0.1 g (80%); R_f (A) 0.67; HPLC K' = 5.85; mp 185-187 °C; $[\alpha]^{20}D$ -56.4; MH^+ 421. Anal. ($C_{25}H_{26}N_2O_4$•TFA) C, H, N (C 71.75, H 6.26, N 6.29).

Example 36

This example describes the synthesis of Boc-Dmt-Tic-NH-tBu.

This intermediate was obtained by condensation of Boc-Dmt-Tic-OH with *tert*-butyl amine via DCC/NMM as described in Example 14: yield 0.26 g (87%); R_f (B) 0.88; HPLC K' = 7.02; mp 153-155 °C; $[\alpha]^{20}D$ -8.2; MH^+ 524; ^{1}H-NMR (DMSO) δ = 1.23-1.44 (m, 18H), 2.16 (s, 6H), 3.05-3.41 (m, 4H), 3.79 (m, 1H), 4.29-4.78 (m, 3H), 6.34 (s, 2H), 6.95 (bs, 1H), 7.14 (s, 4H), 8.22 (bs, 1H).

Example 37

This example describes the synthesis of TFA•H-Dmt-Tic-NH-tBu (**compound 9** in Table II).

Boc-Dmt-Tic-NH-tBu was treated with TFA as described in Example 3: yield 0.12 g (93%); R_f (A) 0.74; HPLC K' = 5.35; mp 150-152 °C; $[\alpha]^{20}D$ -15.6; MH^+ 424. Anal. ($C_{28}H_{33}N_3O_3$•TFA) C, H, N (C 70.89, H 7.85, N 9.92).

Example 38

This example describes the synthesis of TFA•*N,N*-$(Me)_2$-Dmt-Tic-NH-tBu (**compound 14** in Table II).

This *N,N*-alkylated peptide was obtained by exhaustive methylation of TFA•H-Dmt-Tic-NH-tBu (**compound 9** in Table II) as reported in Example 21: yield 0.12 g (88%); R_f

(A) 0.78; HPLC K' = 6.01; mp 157-159 °C; $[\alpha]^{20}D$ –18.7; MH^+ 452. Anal. ($C_{27}H_{37}N_3O_3$•TFA) C, H, N (C 71.81, H 8.26, N 9.30).

Example 39

This example describes the synthesis of Boc-Dmt-Tic-Ala-NH-tBu.

This compound was obtained by condensation of Boc-Dmt-Tic-OH with HCl•H-Ala-OtBu via DCC/HOBt as reported for Boc-Dmt-Tic-Ala-OMe: yield 0.2 g (87%); R_f (B) 0.85; HPLC K' = 8.09; mp 150-152 °C; $[\alpha]^{20}D$ +28.2; MH^+ 595; ^{1}H-NMR (DMSO) δ = 1.23-1.48 (m, 21H), 2.16 (s, 6H), 3.05-3.41 (m, 4H), 3.79 (m, 1H), 4.29-4.78 (m, 4H), 6.34 (s, 2H), 6.95 (bs, 1H), 7.14 (s, 4H), 8.22 (bs, 1H), 8.36 (bs, 1H).

Example 40

This example describes the synthesis of TFA•Dmt-Tic-Ala-NH-tBu **(compound 20** in Table II).

Boc-Dmt-Tic-Ala-NH-tBu was treated with TFA as described in Example 3: yield 0.18 g (91%); R_f (A) 0.64; HPLC K' = 5.35; mp 150-152 °C; $[\alpha]^{20}D$ +25.7; MH^+ 495. Anal. ($C_{28}H_{38}N_4O_4$•TFA) C, H, N (C 67.99, H 7.74, N 11.33).

Example 41

This example describes the synthesis of (R,S)-2-cyano-3-(4-hydroxy-2',6'-dimethylphenyl)-propanoic acid ethyl ester.

Ethyl cyanoacetate (0.62 ml, 7.08 mmol) and, after ten minutes, O-carbethoxy-3,5-dimethyl-4-chloromethylphenol (1.8 g, 7.42 mmol) were added to a solution of sodium ethoxide (Na, 0.17 g, 7.2 mmol; anhydrous EtOH, 12 ml). The reaction mixture was refluxed for 2 hr, cooled and filtered. The solution was evaporated *in vacuo*. The residue was crystallized from H_2O/acetone (5:1, v/v). The product was purified by column chromatography [SiO_2; Et_2O/AcOEt (1:1 v/v)]: yield 1.05 g (60%); R_f (B) 0.74; HPLC K' = 5.38; mp 132-134 °C; MH^+ 248; ^{1}H-NMR (DMSO) δ = 1.25-1.81 (t, 3H), 2.23 (s, 6H), 3.08-3.14 (m, 2H), 4.14-4.27 (m, 3H), 6.44 (s, 2H), 9.17 (s, 1H).

Example 42

This example describes the synthesis of (R,S)-2-cyano-3-(4,hydroxy-2',6'-dimethylphenyl)-propanoic acid.

Sodium hydroxide (1N, 4.68 ml, 4.68 mmol) was added to a solution of (R,S)-2-cyano-3-(4-hydroxy-2',6'-dimethylphenyl)-propanoic acid ethyl ester (1.05 g, 4.24 mmol) in ethanol (10 ml). The reaction mixture was stirred for 24 hr at RT. After evaporation of the solvent, the residue was dissolved in EtOAc and washed with citric acid (10%) and brine. The organic phase was dired and evaporated to dryness. The residue was crystallized from Et_2O/PtEt (1:2, v/v): yield 0.79 g (85%); R_f (B) 0.31; HPLC K' = 3.54; mp 154-156 °C; MH^+ 220.

Example 43

This example describes the synthesis of [des-NH_2-α-cyano-(R,S)-Dmt]-Tic-OtBu.

HOBt (0.09 g, 0.58mmol) and DCC (0.12 g, 0.58 mmol) were added to a solution of (R,S)-2-cyano-3-(4-hydroxy-2',6'-dimethylphenyl)-propanoic acid (0.12 g, 0.53 mmol) and H-Tic-OtBu (0.12 g, 0.53 mmol) in DMF (10 ml) at 0 °C. The reaction mixture was stirred for 3 hr at 0 °C and 24 hr at RT. After evaporation of DMF, the residue was treated as reported for Boc-Dmt-Tic-NH-tetrazole-5-yl: yield 0.18 g (80%); R_f (B) 0.82; HPLC K' = 8.40; mp 140-142 °C; $[\alpha]^{20}D$ –13.85; MH^+ 435.

Example 44

This example describes the synthesis of [des-NH_2-α-cyano-(R,S)-Dmt]-Tic-OH.

[des-NH_2-α-cyano-(R,S)-Dmt]-Tic-OtBu (0.18, 0.42 mmol) was treated with TFA (1 ml) for 0.5 hr at RT. Et_2O/EtPt (1:1, v/v) was added until the product precipitated: yield 0.15 g (96%); R_f (A) 0.68; HPLC K' = 5.55; mp 142-144 °C; $[\alpha]^{20}D$ –15.31; MH^+ 379; IR (KBr) 3420 (OH), 2360 (nitrile), 1636 (C=O , amide), 1734 (C=O, acid), 1142 (C-O, carboxylate anion)
cm^{-1}.

Example 45

This example describes the synthesis of αH(R,S)-Dmt-Tic-OH (**compound 2** in Table II).

HCl (1 N, 4.5 ml) and PtO_2 (0.05 g) were added to a solution of [des-NH_2-α-cyano-(R,S)-Dmt]-Tic-OH (0.15 g, 0.4 mmol) in EtOH (30 ml) and H_2 bubbled for 8 hr at RT. After filtration, the solution was evaporated to dryness. The residue was crystallized from Et_2O/PtEt (1:1, v/v): yield 0.15 g (98%); R_f (A) 0.39; HPLC K' = 2.51; mp 157-159 °C; $[\alpha]_{20}D$ –18.7; MH^+ 383; IR (KBr) 3432 (NH_3^+), 1683 (C=O, amide), 1616 (C=O, carboxylate anion), 1203 (C-O, carboxylate anion) cm^{-1}. Anal. ($C_{22}H_{26}N_2O_4$•TFA) C, H, N (C 69.09, H 6.85, N 7.32).

Example 46

This example describes the synthesis of *N,N*$(Et)_2$-Dmt-Tic-OH (**compound 17** in Table II).

To a stirred solution of TFA.H-Dmt-Tic-OH (0.27 g, 0.56 mM) in CH_3CN (10 ml) was added CH_3CHO (0.147 ml, 4.26 mM) followed by sodium cyanoborohydride (0.829 g, 1.32 mM). Glacial acetic acid (0.11 ml) was added over 10 min and the reaction was stirred at room temperature for 2hr. The reaction mixture was poured into 100 ml of ethyl acetate and then washed with brine. The organic solvent was dried (Na_2SO_4) and evaporated *in vacuo* to give the crude product that was purified by preparative HPLC: yield 0.26 g (90%); R_f 0.73; HPLC K'= 4.18; mp 117-119; [a]D20 -120; MH+ 425. Reference and analytical determinations as in Salvadori et al., *J. Med. Chem.* 40: 3100-3108 (1997).

Example 47

This example describes the binding of the compounds of the present invention to δ-opioid receptors and μ-opioid receptors.

The present inventive compounds were assayed with a rat brain synaptosomal preparation (P_2) that had been preincubated in 0.1 M NaCl, 0.4 mM GDP, 50 mM HEPES, pH 7.5, and 50 g/ml soybean trypsin inhibitor for 60 min at RT to remove endogenous opioids (Lazarus et al., *J. Biol. Chem.* 264: 354-362 (1989)). The assays were conducted as previously described (Salvadori et al. (1995), *supra*; Salavadori et al. (1997), *supra*; and Lazarus et al., *Eur. J. Med. Chem.* 27: 791-797 (1992)). Briefly, the agonists [^{3}H]DPDPE (30-60 Ci/mmol, NEN-DuPont) and [^{3}H]DAGO (30-60 Ci/mmol, Amersham) were used to label δ and μ sites, respectively, under saturation binding conditions (2 hrs at 22 °C). Excess unlabeled peptide (2 μM) established non-specific binding levels and the labeled

membranes were rapidly filtered on Whatman GF/C glass fibre filters, thoroughly washed, dried and measured for radioactivity using CytoScint (ICN, Irvine, CA). The δ antagonist [^{3}H]*N*,*N*-$(CH_3)_2$-Dmt-Tic-OH was catalytically dehalogenated from a diiodo-intermediate to a specific activity of 59.88 Ci/mol and binds to δ receptors with a K_d = 0.39 nM (Kertesz et al., *J. Label. Compds. Radiopharmac.* 41: 1083-1091 (1998)). The receptor binding and biological properties of the unlabeled peptide were previously described (Salvadori et al. (1997), *supra*). All analogues were analyzed in duplicate using 5-9 dosages and at least three independent repetitions using different synaptosomal preparations were conducted for each peptide (actual *n* values are listed in Table II in parentheses) with results given as mean $\pm$ SEM. The affinity constants (K_i) were calculated according to Cheng and Prusoff (*Biochem. Pharmacol.* 22: 3099-3108 (1973)).

As shown in Table II, the elimination of the carboxylate function of H-Dmt-Tic-OH substantially increased the μ affinity of di- and tripeptide derivatives (see **compounds 6-16 and 18-21**). The net effect was the loss of δ-opioid selectivity and the appearance of compounds that were either essentially nonselective (**compounds 6, 9, 10 and 15**), weakly μ selective (**compounds 8, 11 and 16**), or moderately μ selective (**compound 12**). The δ affinity generally remained high for most C-terminally modified analogues with K_i values ranging from 0.07 to 1 nM. Nonetheless, the binding data revealed that several analogues lost high δ affinity, in particular the methyl ester derivative (**compound 8**) and those containing the D-Tic enantiomer (**compounds 12 and 16**).

Interposing a methylene spacer (see **compound 1**) between the C_α of the Tic residue and the carboxylate function (see Table I) to prevent diketopiperazine formation had minimal effect on δ affinity, but enhanced μ affinity. The same chemical approach employing a methylene group between the amino group and the C_α of Dmt (**compound 2**) was more detrimental as compared to H-(R,S)Dmt-Tic-OH. The dipeptide analogues containing hydrazide (**compound 6**), methyl amide (**compound 7**), and tetrazole-5-yl (**compound 10**) exhibited high δ affinities, but each with a marked gain in μ affinity, which was also observed with the Ala-containing tripeptide methyl ester (**compound 19**) relative to its title compound (H-Dmt-Tic-Ala-NH_2).

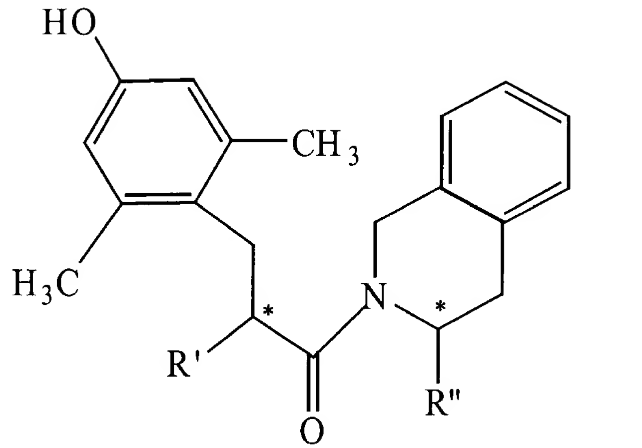
The largest increase in μ affinity occurred in the Dmt-Tic analogues C-terminally substituted with either *tert*-butyl amide (**compounds 9 and 14**) or 1-adamantyl amide (**compounds 11, 12, 15, 16 and 21**). In comparison to H-Dmt-Tic-OH, the μ affinity of

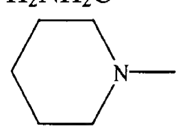
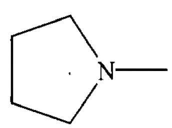
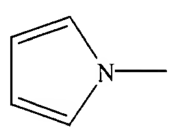
compound 15 rose nearly 2,200-fold relative to *N*,*N*$(Me)_2$-Dmt-Tic-OH. Comparisons between the amidated parental peptides to their C-terminal derivatives, however, indicated smaller changes in μ affinities.

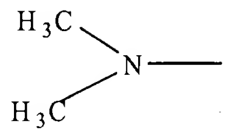
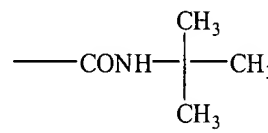
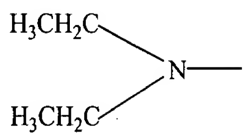
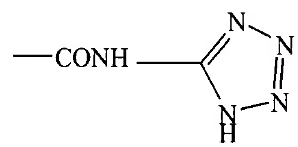
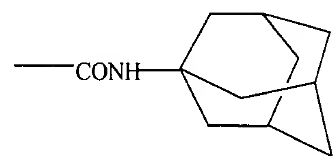
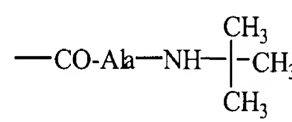
N-alkylation of Dmt-Tic with piperidine-1-yl (**compound 3**), pyrrolidine-1-yl (**compound 4**) or pyrrole-1-yl groups (**compound 5**) (see Table II) decreased δ affinity, particularly the latter compound whose receptor binding was comparable to H-αDmt-Tic-OH (**compound 2**), H-Dmt-Tic-OMe (**compound 8**), and H-Dmt-D-Tic-NH-1-adamantane (**compound 12**). In spite of the bulky N-terminal substituents, the δ selectivities of **compounds 3 and 4** were analogous to other modified peptides.

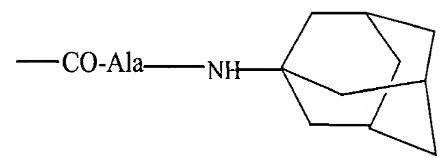
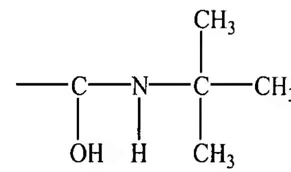
Receptor binding with the δ antagonist $[^3H]$*N*,*N*-$(Me)_2$-Dmt-Tic-OH yielded similar K_i values to those obtained with the agonist $[^3H]$DPDPE in over 80% of the peptides listed in Table II. Exceptions (i.e., peptides whose K_i values differed by at least an order of magnitude) were the title peptide H-Dmt-Tic-OH, which is relatively unstable and forms a diketopiperazine (Marsden et al., *Int. J. Pept. Prot. Res.* 41: 313-316 (1993); Carpenter et al., *J. Am. Chem. Soc.* 116: 8450-8458 (1994); Caspasso et al., *Int. J. Pept. Prot. Res.* 45: 567-573 (1995); and Balboni et al., *Biol. Chem.* 378: 19-29 (1997)) and analogues (**compounds 2, 6, 8 and 10**).

TABLE I



Compound No.	R'	Compound No.	R"
2	H_2NH_2C-	1	—CH_2COOH
3		2-5, 17	—COOH
4		6	—$CONHNH_2$
5		7,13	—$CONHCH_3$

Compound No.	R'	Compound No.	R"
1, 6-12, 18-21	H_2N—	8	—$COOCH_3$
13-16, 22		9,14	
17		10	
		11,12,15,16	
		18	—CO-Ala—$NHCH_3$
		19	—CO-Ala—OCH_3
		20	

Compound No.	R'	Compound No.	R"
		21	
		22	

With respect to Table I, the bold numbers refer to the analogues in Table II. Asterisks denote chiral centers.

TABLE II

MEMBRANE RECEPTOR BINDING OF THE MODIFIED DMT-TIC PHARMACOPHORE

		$K_i\delta$(nM)				
Compound No.	Peptide	[^{3}H]DPDPE	[^{3}H]*N,N*(CH_3)$_2$-Dmt-Tic-OH	$K_i\mu$ (nM) [^{3}H]DAGO	Agonist $K_i\mu/K_i\delta$	Antagonist $K_i\mu/K_i\delta$
	Parental Compounds					
	H-Dmt-Tic-OH	0.022 ± 0.002(6)	0.34 ± 0.05 (4)	3320 ± 435 (7)	150800[a]	9852
	H-Dmt-Tic-NH_2	1.22 ± 0.09 (6)	2.13 ± 0.12 (3)	277 ± 26 (3)	227[a]	130
	H-Dmt-Tic-Ala-OH	0.29 ±0.03 (6)	0.29 ± 0.05 (3)	813 ± 68 (4)	2852[a]	2784
	H-Dmt-Tic-Ala-NH_2	0.24 ± 0.02 (5)	0.69 ± 0.13 (3)	47 ± 3.4 (4)	195[a]	68
	N,N(Me)$_2$-Dmt-Tic-OH	0.12 ± 0.02 (3)	0.07 ± 0.01 (4)	2435 ± 462 (3)	20636[b]	34785
	H-(R,S)Dmt-Tic-OH	0.46 ± 0.001 (3)	0.80 ± 0.19 (4)	1158 ± 327 (3)	2517	1449
	cyclo (DMT-Tic)	9.58 ± 1.98 (3-5)		no activity	635 ± 69 (3-5)	no activity
	Dipeptide Derivatives					
1	H-Dmt-βTic-OH	0.85 ± 0.20 (5)	0.71 ± 0.04 (3)	418 ± 86 (3)	498	590
2	H-αDmt-Tic-OH	11.2 ± 3.5 (3)	475 ± 37 (4)	1740 ± 16 (3)	155	4
3	[des-NH_2-α-piperidine-1-yl]-Dmt-Tic-OH	1.18 ± 0.10 (3)	0.74 ± 0.30 (3)	2039 ± 264 (3)	1728	2755
4	[des-NH_2-α-pyrrolidine-1-yl]-Dmt-Tic-OH	1.62 ± 0.19 (3)	1.42 ± 0.14 (3)	814 ± 65 (3)	502	573
5	[des-NH_2-α-pyrrole-1-yl]-Dmt-Tic-OH	16.6 ± 2.5 (5)	9.94 ± 2.5 (3)	5591 ± 411 (3)	338	562
6	H-Dmt-Tic-NHNH$_2$	0.99 ± 0.04 (3)	42.0 ± 7.9 (5)	85.1 + 7.3 (3)	86	2

Compound No.	Peptide	$K_i\delta$(nM) [^{3}H]DPDPE	$K_i\delta$(nM) [^{3}H]N,N(CH_3)$_2$-Dmt-Tic-OH	$K_i\mu$ (nM) [^{3}H]DAGO	Agonist $K_i\mu/K_i\delta$	Antagonist $K_i\mu/K_i\delta$
7	H-Dmt-Tic-NHMe	0.47 ± 0.09 (3)	1.24 ± 0.15 (4)	85.5 ± 7.7 (3)	182	69
8	H-Dmt-Tic-OMe	9.64 ± 2.2 (3)	500 ± 90 (5)	423 ± 25 (3)	44	0.8
9	H-Dmt-Tic-NH-tBu	0.43 ± 0.07 (5)	0.93 ± 0.15 (4)	5.96 ± 0.82 (4)	14	6
10	H-Dmt-Tic-NH-tetrazole-5-yl	0.70 ± 0.03 (3)	9.75 ± 1.73 (5)	37.0 ± 4.5 (3)	53	4
11	H-Dmt-Tic-NH-1-adamantane	0.26 ± 0.05 (4)	1.01 ± 0.26 (3)	0.76 ± 0.05 (4)	3	0.8
12	H-Dmt-D-Tic-NH-1-adamantane	24.5 ± 4.9 (6)	70.3 ± 7.25 (3)	0.26 ± 0.08 (4)	0.01	0.004
13	*N,N*(Me)$_2$-Dmt-Tic-NHMe	0.54 ± 0.07 (3)	0.28 ± 0.02 (3)	359 ± 62 (3)	669	1268
14	*N,N*(Me)$_2$-Dmt-Tic-NH-tBu	0.61 ± 0.02 (3)	0.11 ± 0.04 (3)	226 ± 21 (3)	369	2132
15	*N,N*(Me)$_2$-Dmt-Tic-NH-1-adamantane	0.16 ± 0.17 (3)	0.12 ± 0.02 (3)	1.12 ± 0.10 (3)	7	9
16	*N,N*(Me)$_2$-Dmt-D-Tic-NH-1-adamantane	140 ± 30 (6)	120 ± 25.9 (3)	50.5 ± 2.6 (3)	0.36	0.42
17	*N,N*(Et)$_2$-DMT-Tic-OH	0.92± 0.19 (3-5)		not tested	35.3 ± 1.96 (3-5)	not tested
	Tripeptide Derivatives					
18	H-Dmt-Tic-Ala-NHMe	0.058 ± 0.01 (3)	0.12 ± 0.04(3)	5.75 ± 0.72 (3)	100	47
19	H-Dmt-Tic-Ala-OMe	0.23 ± 0.09 (3)	0.14 ± 0.04 (3)	11.3 ± 1.87 (3)	48	83
20	H-Dmt-Tic-Ala-NH-tBu	0.066 ± 0.01 (3)	0.08 ± 0.02 (3)	4.03 ± 0.21 (3)	61	48
21	H-Dmt-Tic-Ala-NH-1-adamantane	0.073 ± 0.02 (3)	0.04 ± 0.01 (3)	2.52 ± 0.56 (4)	35	72

With respect to Table II, the numeric value in the parentheses indicates the number (n) of repetitions of independent binding assays using different synaptosomal preparations. [a]Salvadori et al. (1995), supra. [b]Salvadori et al. (1997), supra

Example 48

This example describes the functional bioactivity of the present inventive compounds.

The functional bioactivity of the present inventive compounds was assessed using standard functional bioassays of δ and μ activity in MVD and GPI (Salvadori et al. (1995), *supra*; and Salvadori et al., *J. Med. Chem.* 36: 3748-3756 (1993)). Briefly, a 2-3 cm segment of GPI was placed in a 20 ml tissue bath containing Kreb's solution, 70 μM hexamethonium bromide and 0.125 μM mepyramine and aerated with 95% O_2/5% CO_2 at 36 °C. Transmural stimulation of GPI was by means of a square-wave electrical pulse of 0.5 ms duration at a frequency of 0.1 Hz. A single MVD was suspended in 4 ml modified Kreb's solution aerated with 95% O_2/ 5% CO_2 at 33 °C. An isometric transducer recorded the twitch induced by field stimulation (0.1 Hz for 1 ms at 40 V). Dose-response curves were obtained (Salvadori et al. (1995), *supra*) for both tissues. The μ agonist activity was compared against dermorphin (IC_{50} = 1.82 nM) and δ antagonism was determined through the inhibition of deltorphin C (δ_1 receptor agonist, IC_{50} = 0.54 nM) in comparison to the nonpeptide δ antagonist naltrindole. Data were derived from at least four independent tissue samples and dose-response curves from which the pA_2 values were determined (Salvadori et al. (1997), *supra*) according to Arunlakshana and Schild (*Br. J. Pharmacol.* 14: 48-58 (1959)). In other words, antagonism was expressed as pA_2 (negative logarithm of the concentration that causes 50% inhibition) and agonism was expressed as the IC_{50}.

Dmt-Tic analogues (**compounds 1, 9, 13, 15, 20 and 21**) demonstrated the highest δ-antagonist functional bioactivities (Table III) as well as some of the highest δ-receptor affinities (Table II). Of greater interest, however, was the observation that several analogues acquired unusual bioactivity profiles. For example, both of **compounds 13 and 15** elicited excellent δ antagonism, yet the former was a weak μ antagonist and the latter clearly displayed μ agonism (Table III). Inclusion of the D-Tic enantiomer (**compounds 12 and 16**) essentially greatly reduced bioactivity on MVD with μM activity on GPI. Replacement of the N-terminal amine through alkylation by piperidine-1-yl (**compound 3**),

pyrolidine-1-yl (**compound 4**) or pyrrole-1-yl (**compound 5**) was detrimental for all bioactivity measurements on MVD (Table III). *N*-alkylation by methyl groups to form secondary or tertiary amines was the only N-terminal substitution tolerated (Lazarus et al. (1998), *supra*; see Table III). Interestingly, the non-alkylated, C-terminally modified dipeptides, i.e., **compounds 11 and 12**, lacked δ antagonism; however, **compound 11** surprisingly manifested a weak δ agonism and moderate μ agonism in spite of its high δ affinity (see Table II), while other analogues, i.e., **compounds 7, 13, 14 and 18**, also produced anomalous biological activities on MVD and GPI (see Table III) relative to their receptor binding parameters (see Table II). H-Dmt-Tic-NHMe (**compound 7**) had extraordinarily weak δ agonism with low μ antagonism, while its Ala-tripeptide derivative (**compound 18**) gave very weak δ and μ agonism. Despite the high δ-receptor affinity of **compound 14**, the bioactivity data indicated only modest δ antagonism and weak μ antagonism. The tetrazole-5-yl-amide analogue (**compound 10**) was a weak δ antagonist with very minimal activity on GPI (see Table III).

TABLE III

FUNCTIONAL BIOACTIVITY OF THE MODIFIED DMT-TIC PHARMACOPHORE

		MVD			GPI		
Compound No.	Peptide	pA_2 (range)	K_e(nM)	ED_{50}(μM)	pA_2 (range)	K_e(μM)	ED_{50}(μM)
	H-Dmt-Tic-OH	8.2	5.7	-	-	-	>10[a]
	H-Dmt-Tic-NH_2	7.2	42	-	-	-	>10[a]
	H-Dmt-Tic-Ala-OH	8.4	4.0	-	-	-	>10[a]
	H-Dmt-Tic-Ala-NH_2	8.0	9.0	-	-	-	4.74 ± 0.9
	N,N$(Me)_2$-Dmt-Tic-OH	9.4	0.28	-	-	-	>10[b]
	H-(R,S)Dmt-Tic-OH	8.17 (7.8-8.6)	6.76	-	-	-	>10
	cyclo (DMT-Tic)	5.43			-		
1	H-Dmt-βTic-OH	8.8 (8.6-9.1)	1.41	-	-	-	>10
3	[des-NH_2-α-piperidine-1-yl]-Dmt-Tic-OH	7.31 (7.15-7.47)	49	-	-	-	>10
4	[des-NH_2-α-pyrrolidine-1-yl]-Dmt-Tic-OH	6.9 (6.9-7.1)	121	-	-	-	>10
5	[des-NH_2-α-pyrrole-1-yl]-Dmt-Tic-OH	6.39 (6.0-6.7)	408	-	-	-	>10
7	H-Dmt-Tic-NHMe	-[d]	>10,000	21.2 (9.0-45)	5.94 (4.2-7.6)	1.15	>10
9	H-Dmt-Tic-NH-tBu	8.24 (8.2-8.5)	1.74	-	-	-	1.04 (0.69-1.5)
10	H-Dmt-Tic-NH-tetrazole-5-yl	7.44 (7.1-7.7)	36.3	-	-	-	8.2 (2.7-24.9)

		MVD			GPI		
Compound No.	Peptide	pA_2 (range)	K_e(nM)	ED_{50}(μM)	pA_2 (range)	K_e(μM)	ED_{50}(μM)
11	H-Dmt-Tic-NH-1-adamantane	-	>10,000	0.87 (0.8-1.2)	-	-	0.036 (0.019-0.068)
12	H-Dmt-D-Tic-NH-1-adamantane	-	>10,000	-	-	-	1.68 (1.23-2.3)
13	*N,N*(Me)$_2$-Dmt-Tic	9.39 (8.8-9.9)	0.41	-	6.41 (6.3-6.5)	0.39	>10
14	*N,N*(Me)$_2$-Dmt-Tic-NH-tBu	7.85 (7.5-8.1)	14.1	-	.52 (6.2-6.7)	0.30	>10
15	*N,N*(Me)$_2$-Dmt-Tic-NH-1-adamantane	9.06 (8.6-9.5)	0.87	-	-	-	0.016 (0.011-0.023)
16	*N,N*(Me)$_2$-Dmt-D-Tic-NH-1-adamantane	6.91 (6.8-7.0)	128	-	-	-	4.48 (3.17-6.32)
17	*N,N*(Et)$_2$-Dmt-Tic-OH	not tested			not tested		
18	H-Dmt-Tic-Ala-NHMe	-	-	1.29 (1.18-1.42)	-	-	0.29 (0.18-0.45)
20	H-Dmt-Tic-Ala-NH-tBu	9.16 (8.7-9.5)	0.69	-	6.76 (6.6-6.9)	0.17	>10
21	H-Dmt-Tic-Ala-NH-1-adamantane	9.29 (9.0-9.5)	0.51	-	-	-	1.0 (0.7-3.0)

With respect to Table III, data were derived from at least four (at least three for compounds 17 and 18) independent tissue samples and dose-response curves. pA_2 is the mean and the range is in parentheses. [a]Salvadori et al. (1995), *supra*. [b]Salvadori et al. (1997), *supra*. [c]Temussi et al. (1994), *supra*. [d]A dash indicates the absence of pharmacological activity.

Example 49

This example describes the ability of certain compounds of the present invention to inhibit hMDR-1.

Stably transfected G-185 fibroblasts containing hMDR-1 and NIH 3T3 cell lines were grown to confluent monolayers in DMEM containing 4.5 g glucose/l, 10 % fetal calf serum, L-glutamine, penicillin (50 units/ml), streptomycin (50 μg/ml) and 1 mM sodium pyruvate in 5% CO_2 at 37 °C. G-185 cells were maintained in 60 ng/l colchicine.

The assay for Pgp is based on the diffusion of non-fluorescent calcein AM across the cell membrane. Calcein AM is then hydrolyzed and accumulates as the fluorescent calcein. In transfected cells expressing excess hMDR-1, Pgp extrudes calcein AM from the cell before hydrolysis occurs; inhibition of hMDR-1 results in the accumulation of intracellular calcein. Transfected G-185 cells and the NIH 3T3 cells were analyzed in triplicate using seven graded dosages of verapamil or the opioid peptides in 96-well plates. Cells (2.5 x 10^6/ well) were incubated in 100 μl culture medium for 15 min at 37 °C and 50 μl of 1 μM calcein AM in PBS, plus 50 μl of verapamil or one of the present inventive compounds in PBS, or 100 μl of PBS alone (control) were added. Cells were mixed and incubated for 15 min at 37 °C, then centrifuged for 5 min at 2,000 x g, and then washed three times in 200 μl PBS. Cell pellets were resuspended in 200 μl PBS and transferred to white Microfluor Plates (Nunc, Copenhagen, Denmark) in order to measure calcein-specific fluorescence (494 nm absorption; 517 nm emission). Internal controls were carried out with each experiment using verapamil and PBS in order to compare the consistency between cell preparations. Means $\pm$ SE (error bars) were determined and plotted using Prism ™ (GraphPad, San Diego, CA).

The results are shown in Fig.1A-1D, all of which are graphs of fluorescence vs. concentration (μM). In Fig. 1A, the inhibition of hMDR-1 in G-185 cells (o) and NIH 3T3 cells (•) by verapamil (control) is shown. In Fig. 1B, the inhibition of hMDR-1 in G-185 cells with NIH 3T3 cells (•) by the 1-adamantyl amide derivatives *N,N*(Me)$_2$-Dmt-Tic-NH-1-adamantane **(compound 15)** (o), H-Dmt-Tic-NH-1-adamantane **(compound 11)** (Δ), H-Dmt-Tic-Ala-NH-1-adamantane **(compound 12)** (▲), and *N,N*(Me)$_2$-Dmt-Tic-OH (□) is shown. In Fig. 1C, the inhibition of hMDR-1 in G-185 cells with NIH 3T3 cells (•) by the *tert*-butyl amide derivatives *N,N*(Me)$_2$-Dmt-Tic-NH-*t*Bu **(compound 14)** (o), H-Dmt-Tic-Ala-NH-*t*Bu **(compound 20)** (Δ), and H-Dmt-Tic-NH-*t*Bu **(compound 9)** (▲) is shown. In

Fig. 1D, the inhibition of hMDR-1 in NIH 3T3 cells (•) and with naltrindole (•---•) or in G-185 cells with naltrindole (Δ), cyclo(Dmt-Tic) (o) or *N,N*-$(Et)_2$-Dmt-Tic-OH **(compound 17)** (▲) is shown.

Inhibition of Pgp in G-185 fibroblasts occurred in a dose-response manner with Dmt-Tic dipeptides containing 1-adamantyl amide and with or without *N*-alkylation (Fig. 1B) comparable to verapamil (Fig. 1A). Peptides lacking N- or C-terminal hydrophobic substituents (Fig. 1A, deltorphin B, deltorphin A, DER, DAGO, DPDPE, DAMME, DtMe, DPD, $[Trp^4,Tyr^5]$DER, $[Trp^4,Lys\text{-}OH^7]$DER, DSB, DTA, PipDTOH and MeDTOH) were inactive. The maximum effective dose of the peptides and verapamil fell within the range of 75-100 μM. In contrast, NIH-3T3 fibroblasts lacking membrane Pgp-1 were unresponsive. The spatially confined aromatic rings of Dmt-Tic and the saturated rings of 1-adamantane presented an optimal configuration to support the inhibition of hMDR-1 activity, which is diminished by inclusion of the anion function that determines δ-receptor selectivity. The order of inhibition by 1-adamantyl amide derivatives was: *N,N*$(Me)_2$-Dmt-Tic-NH-1-adamantane **(compound 15)** = H-Dmt-Tic-NH-1-adamantane **(compound 11)** > H-Dmt-Tic-Ala-NH-1-adamantane **(compound 21)** >> *N,N*$(Me)_2$-Dmt-Tic-OH (inactive). These results clearly demonstrate that inhibition of hMDR-1 only occurs in the presence of Dmt-Tic modified with hydrophobic groups. Fortuitously, H-Dmt-Tic-NH-1-adamantane **(compound 15)** only exhibited weak biological activities (Table III) and, thus, may function as a "chemosensitizer" without side effects on the δ- or μ-opioid systems.

Although the 1-adamantyl amide derivative of Dmt-Tic enhanced the inhibition of hMDR-1, incorporation of Ala^3-NH-1-adamantyl-amide curtailed activity (see Fig. 1B). These observations suggest that distance between hydrophobic and aromatic centers in the peptide play a definitive role in the interaction with hMDR-1, and that the inhibitor effectiveness of the Dmt-Tic compounds was independent of their receptor binding activities or pharmacological bioactivites (Table III). A reduction in the hydrophobicity at the C-terminus by the graded reduction in hydrophobicity from 1-adamantyl amide to *tert*-butyl amide and methyl amide (see Fig. 1C) was comparable to the diminution of activity in dipyridamole analogues containing only a single methyl group. The order of activity by *tert*-butyl amide substances was: *N,N*$(Me)_2$-Dmt-Tic-NH-*t*Bu **(compound 14)** > H-Dmt-Tic-Ala-NH-*t*Bu **(compound 20)** > H-Dmt-Tic-NH-*t*Bu **(compound 9)** >> H-Dmt-Tic-NHMe **(compound 7)** (inactive). In the case of *tert*-butyl amide derivatives, the additional

hydrophobicity afforded by *N*-alkylation by methyl groups increased the inhibition of hMDR-1 (see Fig. 1C). While *N,N*$(Me)_2$-Dmt-Tic-OH was inactive, *N*-alkylation by ethyl groups increased the inhibition of hMDR-1, but substantially less than that observed with the *tert*-butyl amide or 1-adamantyl amide conjugates (see Fig. 1D). Interestingly, the elevation in hydrophobicity associated with *N*-alkylation of the Dmt-Tic pharmacophore enhanced δ antagonism *in vitro* by 20-fold without significant changes on δ-opioid binding parameters. On the other hand, 1-adamantyl amide or *tert*-butyl amide increased μ-receptor binding without interfering with δ-receptor interactions to produce non-selective or μ-selective opioid peptides.

The spontaneous formation of the diketopiperazine, cyclo(Dmt-Tic), was a moderately effective inhibitor of hMDR-1 (see Fig. 1D). These results with cyclo(Dmt-Tic) call into question the requirement for a tertiary amine for Pgp inhibition. On the other hand, the non-peptide δ antagonist naltrindole exhibited inhibition that dissipated at high concentrations (see Fig. 1D), which suggests possible disruption of membrane integrity. The lack of inhibition with opioid agonists, such as dermorphin, deltorphin and the enkephalin analogues DAGO, DPDPE and DAMME, indicates that the charged function retards passage through the BBB. Thus, the data indicate that enhancing the hydrophobicity of Dmt-Tic analogues in order to accelerate passage through the BBB also makes them better substrates for Pgp. Specifically, the covalent addition of *tert*-butyl amide or 1-adamantyl amide converts a potent opioid antagonist into an inhibitor of hMDR-1. The fact that H-Dmt-Tic-NH-1-adamantane **(compound 15)** exhibits minimal bioactivity would make it an erstwhile candidate as a chemosensitizer for chemotherapy of cancers containing hMDR-1.

Incorporation by Reference

All sources (e.g., inventor's certificates, patent applications, patents, printed publications, repository accessions or records, utility models, World-Wide Web pages, and the like) referred to or cited anywhere in this document or in any drawing, Sequence Listing, or Statement filed concurrently herewith are hereby incorporated into and made part of this specification by such reference thereto.

Guide to Interpretation

The foregoing is an integrated description of the invention as a whole, not merely of any particular element or facet thereof. The description describes "preferred embodiments" of this invention, including the best mode known to the inventors for carrying it out. Of course, upon reading the foregoing description, variations of those preferred embodiments will become obvious to those of ordinary skill in the art. The inventors expect ordinarily skilled artisans to employ such variations as appropriate, and the inventors intend for the invention to be practiced otherwise than as specifically described herein. Accordingly, this invention includes all modifications and equivalents of the subject matter recited in the claims appended hereto as permitted by applicable law.

As used in the foregoing description and in the following claims, singular indicators (e.g., "a" or "one") include the plural, unless otherwise indicated. Recitation of a range of discontinuous values is intended to serve as a shorthand method of referring individually to each separate value falling within the range, and each separate value is incorporated into the specification as if it were individually listed. As regards the claims in particular, the term "consisting essentially of" indicates that unlisted ingredients or steps that do not materially affect the basic and novel properties of the invention can be employed in addition to the specifically recited ingredients or steps. In contrast, the terms "comprising," "having," or "incorporating" indicate that any ingredients or steps can be present in addition to those recited. The term "consisting of" indicates that only the recited ingredients or steps are present, but does not foreclose the possibility that equivalents of the ingredients or steps can substitute for those specifically recited.

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